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Repeated-Dose and Reproductive/Developmental Toxicity of NTO (3-Nitro-1,2,4-Triazol-5-One) in the Rat

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14. ABSTRACT

Male and female Sprague-Dawley rats were administered NTO in corn oil (0, 31.25, 125, and 500 mg/kg-day) via oral gavage for two weeks prior to mating and for an additional two weeks during mating. Female rats were dosed throughout gestation and for four days following birth. Two male recovery groups (0 and 500 mg/kg-day) were held for four weeks following the four-week dosing period. Reductions in testes and epididymal mass and mass ratios were observed in the 500 mg/kg-day group compared to controls. Sperm counts were also reduced in the 500 mg/kg-day group compared to controls with no motile sperm observed. The reductions in testes and epididymal mass were associated with tubular degeneration/atrophy and hypospermia with cribiform change of the epididymides. Complete recovery was not evident in the 500 mg/kg-day recovery group. No discernable differences were recognized between dose groups for the reproductive parameters monitored. Gross external examinations of the offspring did not indicate that NTO presents a developmental hazard.

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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of TSCA § 790.7 (a) – (d).

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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. The statistical analyses of the data were conducted by the U.S. Army Public Health Command statisticians. It is not known if these analyses were conducted in accordance with Good Laboratory Practice Standards.

2. During the study period, there were two instances where the animal room temperature was outside of the targeted range of 68-79 °F. In addition, there were five instances of the animal room being outside of the targeted humidity range of 30-70%. In each case, the problem was reported to maintenance personnel and rectified as soon as possible so that it did not affect the quality or integrity of the data generated from this study.

3. The test article characterization (purity) was conducted by the manufacturer and it is not known whether the testing was done in compliance with the above regulation.

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20 March 2014
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TOXICOLOGY STUDY NO. 85-XC-0FP4-12
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REPEATED-DOSE AND REPRODUCTIVE/DEVELOPMENTAL TOXICITY OF NTO (3-NITRO-1,2,4-TRIAZOL-5-ONE) IN THE RAT
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1 Summary

1.1 Purpose

The primary objective of this study was to determine the initial reproductive and developmental toxicity of 3-Nitro-1,2,4-triazol-5-one (NTO) through the use of a screening test. This test is not designed to provide complete information on all aspects of reproduction and development and only offers a limited means of detecting post-natal manifestations of prenatal exposure or effects induced during post-natal exposure. The secondary objective of this study was to confirm the effects of repeated-dose exposure to NTO using different exposure durations and dose levels than previously evaluated.

1.2 Conclusions

Daily oral exposure to male and female rats at dosages of 31.25, 125, and 500 mg/kg-d NTO in corn oil for four weeks did not induce compound-related pre-term mortality. Clinical signs of toxicity were mainly limited to bright yellow-colored urine at higher dosages with no changes in body mass, body mass gain, and food consumption compared to controls observed throughout the study period.

Treatment with NTO resulted in reductions in testes and epididymides mass and mass ratios in male rats given 500 mg/kg-d. Microscopic evaluation of these tissues revealed severe degeneration/atrophy of the testicular seminiferous tubules along with moderate to severe hypospermia and cribiform change of the epididymides. Sperm counts were reduced in the high dose group (500 mg/kg-d) with no motile sperm observed. Complete recovery was not evident in the high dose satellite group following a 4-week recovery period. Reductions in sperm counts with no motile sperm were also observed in the male satellite group. In female rats, differences between NTO treated and control animals were observed for brain and spleen, and uterus mass and mass ratios. Absolute brain mass was reduced in the 31.25 mg/kg-d dose group relative to controls. Spleen mass and spleen to body mass ratios were reduced in the 31.25 and 500 mg/kg-d dose groups relative to controls. No treatment-related microscopic findings were observed in female rats.

Under the stated study conditions, oral dosages of up to and including 500 mg/kg-d NTO did not appear to affect reproduction or development in Sprague-Dawley rats. Based upon the gross and microscopic findings in male rats following 4 weeks of treatment, as well as the results of the cauda epididymal sperm analysis, infertility would have likely been observed in the high dose males with an extended pre-mating dosing period. Gross external examinations of the offspring on the day of birth and on day 4 postpartum did not indicate that NTO presents a developmental hazard.

2 References

See Appendix A for a listing of references.

3 Authority

Military Interdepartmental Purchase Request (MIPR) No. MIPRIJDATHR142. This toxicology study addresses, in part, the environmental safety and occupational health requirements outlined in Army Regulations (AR) 200-1, AR 40-5, and AR 70-1; Department of Defense Instruction 4715.4; and Army Environmental Requirements and Technology Assessments (Department of the Army (DA), 2007a and b; DA, 2003; Department of Defense (DOD), 1996; and U.S. Army Environmental Command (USAEC), 2009). It was performed as part of an on-going effort by the U.S. Army Environmental Quality Technology (EQT), Ordnance Environmental Program Pollution Prevention Team, to produce safer ordnance. This program is under the direction of the U.S. Army Research, Development, and Engineering Command (USARDECOM) Environmental Acquisition Logistics & Sustainment Program and EQT Pollution Prevention.

4 Background

As a result of the Department of Defense (DOD)-wide initiative to improve munitions safety, the U.S. Army is developing insensitive munitions (IM) for incorporation into its inventory of conventional ammunition and missiles. The Army's IM Program is dedicated to developing munitions that reliably perform as they are intended but are less prone to inadvertent initiation from external stimuli such as bullet/fragment impact, heat from fire, and shock from neighboring explosions (Duncan 2002). The production of insensitive munitions requires the use of intrinsically insensitive explosives that contribute to lower order responses to inadvertent external stimuli. Despite the slightly lower performance of NTO compared to TNT, there has been a renewed interest in its use in explosive formulations based on the lower sensitivity as a melt-cast medium observed during testing and the less stringent shipping requirements. This has led to the development of a range of melt-castable explosives at Picatinny Arsenal, collectively known as "PAX" explosives (Davies and Provatas, 2006). To support the possible fielding and full-scale production of these PAX explosives, occupational exposure guidelines need to be developed and refined using toxicity data in a mammalian system to assess any occupational health hazards associated with the use and production of this material.

NTO is being investigated as a less sensitive direct replacement for traditional explosives such as TNT and RDX. NTO is a crystalline powder that is one of the components used in the formulation of an insensitive explosive referred to as IMX101. NTO was first reported in 1905 but was not used as an explosive until the early 1980's when it was discovered that the French were developing a "new insensitive explosive", which was later reported to be NTO. Renewed interest in the energetic properties of NTO has been fueled by the need to develop munitions that are less prone to inadvertent initiation during transport and routine handling. The reduced sensitivity to environmental stimuli and nearly equal performance during testing make NTO-based formulations desirable replacements for currently fielded munitions (Spear et al, 1989; Smith and Cliff, 1999). In addition to minimizing collateral damage from weapon or ordnance accidents, insensitive munitions offer logistical advantages on the battlefield. As modern battlefields increasingly shift into populated urban centers, insensitive munition inventories represent a less desirable target for

terrorists and minimize the threat to surrounding communities. Less sensitive munitions could also potentially be more cost effective and efficient to transport if granted reduced DOD/Department of Transportation (DOT) hazard classification rankings (DA, Rapid Action Revision (RAR) 2009).

A literature search was conducted prior to the initiation of the study revealing a limited amount of preliminary toxicity data. The reported mouse and rat oral LD_{50} values were both greater than 5000 milligrams per kilogram (mg/kg). In addition, NTO was reported to be a mild skin and eye irritant but was not a dermal sensitizer (Los Alamos National Laboratory, 1985). A subchronic oral toxicity study in rats on NTO was performed by this Command in 2008. The subchronic study on NTO revealed changes in both testes and epididymides mass and mass ratios at dosages of 315 mg/kg-day and above. Reductions in sperm counts were also noted at dosages of 315 mg/kg-day and above. Histopathology performed on the 90-day tissues revealed increased incidences of testicular hypoplasia at dosages of 315 mg/kg-day and above (USAPHC (Provisional), 2010). A repeated-dose 14-day range finding study was also completed prior to the initiation of the 90-day study. In this 14-day study, testes mass and mass ratios were reduced compared to controls in male rats administered 500 mg/kg-day NTO and above. No dose group differences were observed for epididymus mass or mass ratios compared to controls and histopathology was not performed on any tissues from the 14-day study. The data from the oral subchronic study was used to calculate an occupational exposure level (OEL) for NTO. In addition to determining if the observed testicular hypoplasia and aspermia significantly affected reproduction, the data from this study will also be used to refine the established OEL that was previously extrapolated from oral toxicity data.

The following table identifies the critical dates of this study.

Table 1. Critical Study Events

Critical Event	Date of Event
Animal Use Protocol Approved	March 30, 2012
Animals Born In-House Transferred to Protocol	April 9, 2012
Study In-Life Initiation	May 7, 2012
Male Necropsies	June 4 & 5, 2012
Pup Gross External Examinations	June 17-30, 2012
Female Necropsies	June 18–July 1, 2012
Recovery Male Necropsies and Experimental Completion	July 2, 2012
Study Completion	March 2014

5 Materials

5.1 Test Substance

NTO is a light green to white crystalline solid with no odor. The chemical formula is $C_2H_2N_4O_3$ and the molecular weight is 130.06 grams per mole (g/mole). The manufacturer has stated that NTO has a melting point of 268-271 °C and a specific gravity of 1.93 (BAE Systems, 2007). The material was supplied by Ordnance Systems, Inc., Kingsport, Tennessee and identified as lot number BAE11L375-061 and batch number 10NTO11-5. The compound purity was performed by the manufacturer and reported as 99.48% pure by high-performance liquid chromotography analysis. The material was shipped to the U.S. Army Research, Development Engineering Command, Engineering Directorate, Pyrotechnics Team, APG-EA, MD 21010. A separate dosing

solution/suspension was prepared for each dose group at targeted concentrations of 6.25, 25, and 100 milligrams/milliliter (mg/ml). Dosing solutions/suspensions were prepared in volumes sufficient for approximately two-three weeks of dosing, resulting in preparation of two sets of dosing solutions. A third batch of 100 mg/ml dosing suspension was also required due to the additional volume needed for dosing of the recovery male animals. For each dosing solution/suspension, the calculated amount of NTO was weighed and placed in a ceramic mortar. The NTO was then wetted with a measured amount of corn oil and ground with a mortar and pestle to a fine consistency. The slurry was transferred to a volumetric flask and the mortar was rinsed with a measured amount of corn oil to remove any remaining slurry. The remaining corn oil was then added to the suspension to achieve the calculated concentration. A one milliliter (ml) sample was taken from each dosing solution/suspension prepared for the study and analyzed using an HPLC with ultraviolet detection to verify the concentration. In addition, the homogeneity of the solutions/suspensions was verified by determining the concentration of samples taken from the top, middle, and bottom of the highest concentration (100 mg/ml) suspension. Samples were collected from a representative suspension (6 mg/ml) at weekly intervals for an eight-week period to determine the stability of NTO in corn oil.

5.2 Animals[*†]

Each phase of this study was conducted using young adult male and female Sprague-Dawley rats born in-house from a stock of timed-pregnant animals obtained from a Charles River Laboratories, Wilmington, Massachusetts. The animal room was maintained at 70.8 ± 0.87 °F and 51.0 ± 8.71% relative humidity during the acclimation and study periods. A certified pesticide-free rodent chow (Harlan Teklad®, 8728C Certified Rodent Diet) and drinking quality water were available *ad libitum* except during overnight fasting prior to final blood collection. Rats were housed individually in suspended polycarbonate boxes with ALPHA-dri® bedding except during the mating period when the animals were housed on elevated wire racks in the polycarbonate boxes to allow for the observation of sperm plugs. Each rat was uniquely identified by number using cage cards. In addition, the animal identification number was recorded on the tail of each rat with a water-insoluble marker. (Teklad® Certified Rat Diet is a registered trademark of Harlan, Teklad. ALPHA-dri® is a registered trademark with Shepherd Specialty Papers.)

5.3 Contract Studies

Tissues from this study were preserved, packaged and transported to U.S. Army Medical Research Institute for Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD, for processing, slide preparation and staining. Slides were returned to USAPHC for evaluation by an American College of Veterinary Pathology board certified military veterinary pathologist.

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[*] Research was conducted in compliance with DoD and federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC 1996.

[†] The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

5.4 Quality Assurance

The AIPH Quality Systems Office audited critical phases of this study. Appendix B provides the dates of these audits, the phases audited, along with the dates that the results of the inspections were reported to the Study Director and Management.

5.5 Study Personnel

Appendix C contains the names of persons contributing to the performance of this study.

6 Methods

6.1 General Description

The study consisted of a repeated-dose oral toxicity test as well as a reproductive/developmental screening test and was conducted in a manner consistent with the methods outlined in the Organization for Economic Cooperation and Development (OECD) Test Guideline 422 (OECD, 1996). Neurobehavioral observations were omitted from this study due to the negative results obtained during the performance of a subchronic oral toxicity study on NTO previously performed by this Institute (USAPHC (Provisional), 2010). Dose selection was based on the findings obtained from previously-performed 14- and 90-day oral toxicity studies with the expectation of producing reproductive effects at the highest dose.

6.2 Test Substance Administration

Forty male and female Sprague Dawley rats, eight weeks old, weighing 339.8 ± 21.78 and 215.5 ± 12.82 grams, respectively, at the start of dosing were used for this study. Since these animals were born in-house and transferred for use on the study, they were acclimated for a 4-week period until they reached the appropriate age to be used. The animals were weighed and observed periodically throughout the acclimation period to assess their overall health. Following the acclimation period, ten rats of each sex were randomly distributed, according to body mass, into three NTO treatment groups and a corn oil control group. Dosage levels were set at 31.25, 125, and 500 mg/kg-day. Male and female rats were assigned to evenly distributed experimental start dates to facilitate the scheduling of necropsies. The volume of dosing solution/suspension per kilogram of body mass was equivalent across dose groups (5 ml/kg), including corn oil control animals. Animals were dosed daily (7 days/week) using a stainless steel 16 gauge x 2 inch gavage needle.

Male rats were dosed for a total of four weeks encompassing the two-week pre-mating period and the two-week mating period. Female rats were dosed throughout the study including the two-week pre-mating period, the variable time to conception, the duration of pregnancy, and up to and including postpartum day four. Dose was determined by the most recent weekly body mass measurement.

In addition to the main study animals, twenty male Sprague Dawley rats, eight weeks old, weighing 337.8 ± 28.90 grams at the start of dosing, were used as a satellite group to detect delayed occurrence, persistence of, or recovery from the toxic effects of NTO. These animals were from the

same stock of animals used for the main study and were randomly distributed, according to body mass, into a 500 mg/kg-day NTO treatment group and a corn oil control group consisting of ten male rats each. Satellite animals were dosed concurrently with main study animals seven days/week for a period of four weeks and were then held, but not dosed, for an additional four-week period prior to necropsy. The volume of NTO dosing suspension and corn oil per kilogram of body mass was equivalent to that of the main study animals (5 ml/kg) with dose determined by the most recent weekly body mass measurement.

6.3 Mating Procedure

Since the animals used for this study were born in-house, the male and female mating pairs for each dose group were randomly assigned according to dam to eliminate the possibility of inbreeding. Following the two-week pre-mating period, male and female rats were pair-housed in solid-bottom cages with elevated wire racks placed in the bottom. The female rats remained pair-housed with the same male rat until a sperm or vaginal plug was observed or a period of two weeks elapsed. The female rats and the cages were examined each morning prior to dosing for the presence of a vaginal or sperm plug. Day 0 of pregnancy was defined as the day a vaginal plug was observed and the pair was separated to be placed back in individual cages.

6.4 Body Mass and Food Consumption

Male and female rats were weighed several times during the acclimation period, on the first day of dosing, and weekly thereafter. During pregnancy, female rats were weighed on gestational days 0, 7, 14, 20, within 24 hours of parturition, and on day 4 postpartum. Female rats showing no evidence of copulation resumed their normal weekly weigh schedule following the 2-week mating period. Weekly body mass was obtained from satellite male animals during both the exposure and recovery periods. Terminal body mass was obtained the morning of necropsy following overnight fasting for all animals. Litters were weighed within 24 hours of parturition (day 0 or 1 postpartum) and on day 4 postpartum.

Feed was provided *ad libitum* seven days per week in weighed feeder bins with the exception of overnight fasting prior to necropsy. Feeder bins were reweighed on the same days body mass was obtained. Grams of food consumption for each period were calculated by subtracting the mass of the empty feeder from the mass of the full feeder. Food consumption was not monitored during the mating period due to pair-housing.

6.5 Observations

A thorough physical examination of each animal, including the male satellite group, was performed each day concurrently with the dosing procedure. Observations for mortality and signs of toxic effects were made twice daily, once in the morning following dosing and once in the afternoon, except on weekends when observations were only performed in the morning. Observations included, but were not limited to, evaluation of the skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects (e.g., salivation), central nervous system effects (e.g., tremors and convulsions), changes in the level of activity, gait, and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or changes in behavior (e.g., self-mutilation). Pregnant females approaching gestation day 21 were observed more frequently to

allow for an accurate determination of gestation duration. One-time functional observations were omitted from this study due to the negative results obtained from a previously-performed subchronic oral toxicity study on NTO in rats.

Each litter was examined within 24 hours of parturition to establish the number and sex of live pups, litter mass, stillbirths, runts, and the presence of gross abnormalities. Litters were again examined on postpartum day 4 to establish the number and sex of live pups, litter mass, bizarre behavior, and the presence of gross external abnormalities.

6.6 Necropsy

Rats that died during the course of this study were submitted for gross necropsy. Tissues that were not grossly autolytic were submitted for histopathological evaluation. Following the appropriate treatment period, all surviving adult rats were anesthetized with carbon dioxide (CO_2), blood was collected by intracardiac puncture, and rats were euthanized using CO_2. Surviving male rats were euthanized following four weeks of treatment while surviving female dams were euthanized on postpartum day 5. Male necropsies were scheduled over two days based on the staggered experimental start dates and dam necropsies were based on their delivery date. Female rats that did not become pregnant were euthanized 24-25 days following the last day of the mating period. Litters were examined, anesthetized with CO_2, and euthanized on post-natal day 4. Male rats in the satellite group were euthanized 28 days following the last day of their treatment period. A macroscopic examination was conducted on all terminal animals, noting all lesions and abnormal observations. The following organs and tissues, or representative samples, were preserved in a suitable medium: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid/parathyroid; thymus; lungs and trachea; pharynx; larynx; nose; heart; femur bone marrow; salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar) and exorbital lachrymal glands. In addition, the following organs were removed, trimmed in a uniform manner, and weighed: liver; kidneys; adrenals; gonads; spleen; brain; epididymides; uterus; thymus, and heart.

6.7 Sperm Analysis

Cauda epididymal sperm counts were determined on all male rats using a computer assisted sperm analyzer (TOX IVOS-CASA, Hamilton Thorne Research, Beverly, MA). After removal, trimming, and weighing, one epididymis was further trimmed to select the cauda portion, weighed, placed in one well of a six-well plate containing 10 ml of Roswell Park Memorial Institute-1640 (RPMI-1640; Sigma-Aldrich, St. Louis, MO) medium at approximately 37 °C and minced using two scalpel blades to release sperm. The suspension was incubated for 15 minutes at approximately 37 °C. Following a brief mixing with a pipette tip, 0.5 ml of suspension was transferred to one well of a six-well plate containing 2 ml of RPMI-1640 medium and stirred again. A chamber of a 100 micron standard count analysis slide (Leja®, Spectrum Technologies, Healdsburg, CA) was loaded with the resulting diluted semen suspension and the slide loaded into the semen analyzer. The number of sperm, number of motile sperm, and number of progressive sperm were determined in duplicate for each animal. The data were expressed as millions of sperm per ml of suspension and millions of sperm per gram cauda epididymis.

6.8 Clinical Chemistry and Hematology

Blood was obtained from CO_2 anesthetized adult animals via intracardiac puncture at the termination of the study. Blood for clinical chemistry analyses was transferred to tubes with no anticoagulant, allowed to clot for at least 20 minutes, and centrifuged to obtain serum. Blood for hematology analyses was transferred immediately to tubes containing tripotassium ethylenediamine-tetraacetic acid (K_3EDTA). Animals were fasted overnight prior to blood collection.

Clinical Chemistry parameters including: albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose (non-fasting) (GLU), globulin (GLOB), lactate dehydrogenase (LDH), inorganic phosphorous (PHOS), total bilirubin (TBIL), total protein (TP), sodium (Na), potassium (K), and chloride (Cl) were determined (VetTest 8008 Chemistry Analyzer; VetLyte Na, K, Cl Analyzer, IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092) on all valid serum samples.

Hematology parameters including: white blood cell count (WBC), WBC differential (% neutrophils (NEU %N), % lymphocytes (LYM %L), % monocytes (MONO %M), % eosinophils (EOS %E), % basophils (BASO %B)), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV) were determined (Cell-Dyn 3700 Hematology Analyzer, Abbott Laboratories, Abbott Park, IL 60064) on all valid samples.

6.9 Histopathology

Tissues were appropriately preserved in 10% buffered formalin, selectively trimmed and placed in cassettes labeled with protocol number, animal identification number, and laboratory-assigned accession number. Testes and epididymides were preserved in modified Davidson's fixative for a period not exceeding 24 hours and were then transferred to 70% ethyl alcohol. Cassettes were placed in labeled formalin-filled bottles and transported to USAMRICD for processing. Tissues were routinely processed and paraffin embedded. All processed and embedded tissues were microtomed at 5 µm thick and automatically stained with hematoxylin and eosin and coverslipped. The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevalence and severity of findings were graded as compared to controls. Findings were assigned as none, minimal, mild, moderate, or severe.

6.10 Statistical Analysis

Analyses were conducted for males and females separately. SPSS 16.0 was used to perform all analyses and statistical significance was defined as $p \leq .05$. For one-time measurement variables in adult animals (hematology, clinical chemistry, organ to body/brain mass ratios, sperm counts, litter/pup parameters), the dose groups were compared using a one-factor analysis of variance (ANOVA). If the dose group effect was significant, a Tukey post hoc test was used to compare pairs of dose groups. The Levene's test was used to determine the variance of the groups. The Tukey post hoc test was used because group variances were equal. Data was checked for normality by plotting residuals. If data was not normal it was natural log transformed. If

transformation still did not satisfy ANOVA assumptions, a non-parametric Kruskal-Wallis (K-W) test was used to analyze dose group differences. (SPSS® is a registered trademark of SPSS, Inc.)

For absolute organ mass, comparison of the dose groups was made using an analysis of covariance (ANCOVA), with body mass at the end of the study as the covariate. Even though the dose groups were assigned at Day 0 to keep the average starting mass for each dose group similar, the average mass could have changed during the study dependent on the dose group. The ANCOVA adjusted for any differences in terminal body mass among the dose groups because heavier animals would tend to have heavier organs. If the dose group effect was significant, a Sidak post hoc test was used to compare pairs of dose groups and dose groups to the control group.

Repeated-measure variables for adult animals (body mass and food consumption) were compared using repeated measures ANOVA. If the dose effect in the ANOVA was significant, a Tukey post hoc test was used to compare pairs of dose groups. If the interaction effect of week and dose group was statistically significant, weekly means were compared but overall dose group means were not because results would have been inconclusive. Verification of normally distributed data (residual plots) and equal variances among dose groups (Levene's test) assumptions was performed.

The distributions of reproductive characteristics were compared descriptively. The sample sizes for each group were too small to test statistically. To test for differences in the count distribution, a chi-square test would have been appropriate. However, given the sample size and the high proportion of females achieving the various reproductive endpoints, the expected value of many of the cells would have been less than 5 and violated the assumptions of a chi-square test.

7 Results

7.1 Analytical Results

The analytical chemistry results are summarized in Tables 2 and 3. The results of the 7-week stability study indicated that the NTO concentration in corn oil remained within acceptable ranges. Weekly recovery percentages ranged from 100-107% throughout the sampling period. Homogeneity testing of the most concentrated NTO/corn oil suspension (100 mg/ml) yielded 92% recovery at the top and 89% recovery at the middle and bottom of the container. Verification of the dosing solution/suspension concentrations prior to use yielded recovery percentages ranging from 85-102% of the nominal concentrations for all batches mixed. Given the concentrated nature of these mixtures and the acceptable limits of the analytical laboratory control samples for this method, these analytical results were considered acceptable. All of the dosage levels are reported using the nominal concentrations.

Table 2. Stability and Homogeneity Results

Nominal Concentration (mg/ml)	Analytical Concentration (mg/ml)
6 (day 0 stability)	6.3
6 (day 7 stability)	6.1
6 (day 14 stability)	6.0
6 (day 21 stability)	6.3
6 (day 28 stability)	6.3
6 (day 35 stability)	6.3
6 (day 42 stability)	6.4
6 (day 49 stability)	6.3
100 (homogeneity top)	92
100 (homogeneity middle)	89
100 (homogeneity bottom)	89

Table 3. Dosing Solution/Suspension Concentration Results

Nominal Concentration (mg/ml)	Analytical Concentration (mg/ml)		
	Batch 1	Batch 2	Batch 3
6.25	6.4	5.9, 5.7, 5.7 (repeats)	
25	23	22, 22, 23 (repeats)	
100	96	87, 85, 90 (repeats)	86

7.2 Clinical Observations and Mortality

Daily oral administration of NTO suspended in corn oil for the selected time periods did not induce compound-related mortality at dosages of 500 mg/kg-d and below. One male rat in the 31.25 mg/kg-d dose group was found dead in its cage on day 18 of the study. This animal did undergo a gross pathological examination but the severity of tissue autolysis prevented the determination of a cause of death. This pre-term mortality was not considered test material related since the animal exhibited no clinical signs of toxicity prior to death.

Very few clinical signs were observed that could directly be attributed to NTO administration and were mainly limited to bright yellow-colored urine at dosages of 125 mg/kg-d and above. Additional signs noted throughout all dose groups that were likely caused by the stress associated with the daily oral gavage procedure included barbering, chromodacryorrhea, and dried red material around nose. Several female and recovery male animals had sores either behind the ears or on the ventral cervical area. The Attending Veterinarian examined the animals and noted localized alopecia with superficial lacerations. The affected animals were treated daily with a topical povidone swab. Although a skin scrape was recommended to determine whether the sores were caused by a parasitic problem or contact dermatitis, the lacerations resolved with topical treatment prior to the skin scrape being performed. Several female animals also exhibited signs resulting from parturition (e.g., blood-stained fur on urogenital area and rough haircoat). Due to the viscosity of the dosing suspensions, some male and female animals throughout all NTO dose groups appeared to have

corn oil around their mouths following dosing. This observation was noted in case the animal aspirated part of the dose and could have developed congested breathing on the following day (see Appendix D).

7.3 Body Mass

No differences in the body mass measurements for any male (including recovery males) or female dose group compared to control animals were observed throughout the study period. Body mass for the male and recovery male control groups was slightly higher by the end of the study period but the difference was not enough to be statistically significant. Female inter-dose group variability was obviously greater due to the pregnancies (see Appendix E).

7.4 Body Mass Gain and Food Consumption

No differences in body mass gain or food consumption for any male (including recovery males) or female dose group compared to control animals were observed throughout the study period. Female body mass gain and food consumption was reduced in the 500 mg/kg-d dose group during the 2-week pre-mating period compared to the 31.25 mg/kg-d dose group only ($p=0.008$ and 0.009, respectively). No differences were observed between any of the female dose groups during the gestational or postpartum study periods (see Appendices F & G).

7.5 Organ Mass and Ratios

In male rats, differences in organ mass and organ mass ratios were limited to the reproductive organs. Testes and epididymides mass, organ to body mass, and organ to brain mass ratios were decreased in the 500 mg/kg-d dose group compared to all other dose groups, including controls ($p=0.00$ for all). Testes and epididymides mass, organ to body mass, and organ to brain mass ratios remained decreased in the 500 mg/kg-d recovery male dose group compared to recovery controls following a 4-week recovery period ($p=0.00$ for all). In female rats, differences between NTO treated and control animals were observed for brain, spleen, and uterus mass and mass ratios. Absolute brain mass was reduced in the 31.25 mg/kg-d dose group relative to controls ($p=0.020$). Spleen mass and spleen to body mass ratios were reduced in the 31.25 ($p=0.035$ and $p=0.031$) and 500 mg/kg-d ($p=0.008$ and $p=0.005$) dose groups relative to controls. Following the removal of one outlier in the 500 mg/kg-d dose group, absolute uterus mass and uterus to brain mass ratios were reduced in the 125 mg/kg-d dose group relative to the 500 mg/kg-d group ($p=0.027$ and $p=.016$, respectively) (see Appendix H).

7.6 Sperm Analysis

The number of sperm per gram in the cauda epididymis in male rats in the 500 mg/kg-d group was reduced to 6.8% of the number of sperm per gram found in the controls ($p=0.00$). No motile sperm were found in any of the animals in the 500 mg/kg-d group. Average numbers of sperm per gram were only reduced to 91.7 and 87.2% of control averages in the 31.25 and 125 mg/kg-d dose groups, respectively. Following the 4-week recovery period, the number of sperm per gram in the cauda epididymis of male rats in the recovery 500 mg/kg-d group was reduced to 28.6% of the number of sperm per gram found in the recovery controls ($p=0.00$). No motile sperm were found in any of the animals in the recovery 500 mg/kg-d group (see Appendix I).

7.7 Clinical Chemistry

Differences between NTO treated male and female dose groups and their controls were not present for any of the serum clinical chemistry parameters. Total bilirubin (TBIL) was increased in the male 500 mg/kg-d dose group compared to the 125 mg/kg-d group only (p=0.023). The male TBIL group means did not exhibit any dose-dependent trends and all remained within normal ranges (Charles River Laboratories, 2006). No differences in clinical chemistry parameters were observed between the recovery male 500 mg/kg-d group and the recovery male controls. Phosphorus (PHOS) was elevated in the female 500 mg/kg-d group compared to the 125 mg/kg-d group (p=0.022) but all group means were well above normal ranges (Charles River Laboratories, 2006) (see Appendix J).

7.8 Hematology

Differences between NTO treated male and female dose groups and their controls were not present for any of the hematology parameters. Mean cell volume (MCV) and mean cell hemoglobin (MCH) were increased in the male 500 mg/kg-d group compared to the 31.25 mg/kg-d group (p=0.041 and p=0.010, respectively). All male MCV values remained within normal ranges while only the male 31.25 mg/kg-d MCH means were within normal ranges. MCH means for the control, 125, and 500 mg/kg-d groups were all above historical ranges. Mean platelet counts (PLT) were decreased in the male 500 mg/kg-d group compared to the 125 mg/kg-d male means but all group means remained within normal ranges. No differences in hematology parameters were observed between the recovery male 500 mg/kg-d group and the recovery male controls. Percent basophils (BASO %) were elevated in the female 500 mg/kg-d group compared to the 125 mg/kg-d group (p=0.015), however, all group means were well above normal ranges (Charles River Laboratories, 2006) (see Appendix K).

7.9 Prothrombin Time

No differences were observed with respect to prothrombin time among any of the male (including recovery males) or female dose group means (see Appendix L).

7.10 Reproductive/Developmental Parameters

No discernible differences were recognized among the dose groups, including controls, for the reproductive endpoints expressed as proportions. These endpoints included number of females showing evidence of copulation, number of females achieving pregnancy, number of dams with live young born, and number of dams with live young at postpartum day 4. The sample sizes were too small and proportions were too similar to allow for a meaningful statistical comparison. Of the 10 female rats pair-housed per dose group, 2 out of 10 were not pregnant from the corn oil control and 500 mg/kg-d NTO dose groups. In the control group, a sperm plug was found for one of the mating pairs but not the other. Sperm plugs were observed for both non-pregnant females in the 500 mg/kg-d dose group. One out of 10 females was not pregnant in the 31.25 mg/kg-d dose group and a sperm plug was observed for this pair. All females were pregnant in the 125 mg/kg-d dose group. Historical control data for developmental and reproductive toxicity studies using the Crl:CD® Sprague-Dawley rat indicated that, on average, 92.93 ± 11.1% of the females pair-housed successfully mated. Of those females that successfully mated, 95.72 ± 6.65% were pregnant. Overall, 88.78 ± 11.8% of the female rats pair-housed became pregnant (Charles River

Laboratories, 1993). NTO-treated animals in this study did not exhibit a reduction in pregnancy rates and were within historical averages. (CD® is a registered trademark of Charles River Laboratories)

Dose group averages for the number of days pair-housed prior to finding evidence of copulation, gestational length, pre-implantation loss, pre-natal loss, and post-natal loss were analyzed using a non-parametric Kruskal-Wallis test and were not statistically different (p=0.52, p=0.796, p=0.575, p=0.744, and p=0.431, respectively). Statistical analysis of the number of live pups at birth and at postpartum day 4, the pup sex ratio at birth and at postpartum day 4, the average litter mass at birth and at postpartum day 4, and the number of pup abnormalities (including stillbirths) at birth did not reveal any differences among dose group averages (p=0.974 and p=0.724, p=0.458 and p=0.381, p=0.525 and p=0.898, p=0.573, respectively). A summary of the litter parameters which had historical control data are presented in Table 4. The percent postimplantation loss, number of stillborn pups per litter, and the number of dead pups occurring on post-natal days 1-4 were elevated in all dose groups compared to historical control averages but were the most elevated in the corn oil control group. The average pup mass at birth was also slightly decreased in the corn oil control group compared to the NTO-treated groups and historical controls (see Appendix M).

Table 4. Summary of Litter Parameters

Mean ± S.D.	Corn Oil Control	31.25 mg/kg	125 mg/kg	500 mg/kg	Historical Controls*
Length of Gestation (Days)	22.0 ± 0.00	22.1 ± 0.35	22.0 ± 0.47	22.0 ± 0.00	22.42 ± 0.53
# Implants/Female	15.3 ± 3.01	15.6 ± 2.60	15.5 ± 2.07	15.3 ± 2.25	15.51 ± 1.85
% Postimplantation Loss	14.8 ± 16.88	12.7 ± 8.82	12.5 ± 16.19	8.1 ± 7.89	8.13 ± 3.35
# Live Pups/Litter	12.8 ± 3.28	13.4 ± 2.35	13.4 ± 2.95	13.4 ± 2.13	14.14 ± 1.42
# Stillborn/Litter	1.8 ± 2.71	0.2 ± 0.44	0.4 ± 0.70	0.4 ± 0.74	0.24 ± 0.26
Dead Pups, PND 1-4[#]	3.3 ± 6.04	0.3 ± 0.71	3.2 ± 6.23	2.4 ± 5.13	0.41 ± 0.39[+]
Sex Ratio (% Male)	45.3 ± 12.16	54.8 ± 13.80	54.4 ± 13.52	50.5 ± 15.32	49.68 ± 3.90
Birth Mass (Avg. All Pups)	5.9 ± 0.65	6.4 ± 0.61	6.2 ± 0.70	6.1 ± 0.89	6.33 ± 0.29

* (Charles River Laboratories, 2006)
[+] Historical controls reported as dead pups, PND 1-21
[#] Includes pups humanely euthanized due to total neglect by dam.
PND=post-natal day

7.11 Pathology

A gross pathological examination of the male pre-term mortality in the 31.25 mg/kg-d group was not possible due to the extent of tissue autolysis. In male rats, liver findings included 3 with a pale liver (one control and two 31.25 mg/kg-d animals) and 18 with a dark/mottled liver (three control, four 31.25 mg/kg-d, four 125 mg/kg-d, and seven 500 mg/kg-d animals). Three male rats per dose group were reported to have dark kidneys with the exception of the 31.25 mg/kg-d group with two.

A number of male rats throughout all dose groups were noted as having pale intestines either with or without yellow fluid present (five controls, five 31.25 mg/kg-d, eight 125 mg/kg-d, and eight 500 mg/kg-d animals). Three male controls, two 31.25 mg/kg-d, one 125 mg/kg-d, and two 500 mg/kg-d animals were observed with dark and/or enlarged spleens. One additional male in the 500 mg/kg-d group had a pale spleen and one in the 125 mg/kg-d group had a small spleen. All male animals in the 500 mg/kg-d group were noted as having small testes. Additional male gross pathology findings included one control with a small left testes and one 31.25 mg/kg-d animal with 2 pale brown 1 millimeter areas on the left lobe of the thymus.

In recovery male rats, six rats in both the control and 500 mg/kg-d groups were noted to have dark livers and five animals had dark spleens (three control and two 500 mg/kg-d animals). One control and three 500 mg/kg-d recovery males were observed with dark/mottled kidneys. Eight out of ten males in the 500 mg/kg-d recovery group were noted as having small testes. Additional recovery male gross pathology findings included one control with enlarged submandibular lymph nodes, one control with hydronephrosis of right kidney, and one 500 mg/kg-d male with hydronephrosis of right kidney.

In female rats, liver findings included 7 with a dark liver (three control, one 31.25 mg/kg-d, two 125 mg/kg-d, and one 500 mg/kg-d animals), one 125 mg/kg-d animal with a pale brown liver, and one control with a pale liver. Kidney findings included 4 with dark kidneys (two control, one 125 mg/kg-d, and one 500 mg/kg-d animals) and one 125 mg/kg-d female with left and right cystic kidneys. A number of female rats throughout all dose groups were noted as having pale intestines either with or without yellow fluid present (five controls, nine 31.25 mg/kg-d, eight 125 mg/kg-d, and seven 500 mg/kg-d animals). One female control and one 500 mg/kg-d female were observed with dark spleens. One female control had a 9 x 4 millimeter diverticulum on serosal side of the greater curvature of the stomach, 2 females had distended stomachs full of bedding (one 125 mg/kg-d and one 500 mg/kg-d animals), and one 500 mg/kg-d female had yellow fluid present in the stomach. Female lung findings included one control with red spots on the lung and two 125 mg/kg-d animals with mottled lungs. Additional female gross pathology findings included a scab on ventral chin, a mass in the fat near the ovaries, yellow-tinged mesenteric lymph nodes, enlarged submandibular lymph nodes, thin appearance, yellow-tinged subcutaneous fat, fluid-filled uterus, dilated uterus, yellow-stained fur, and a gas distended cecum. Gross pathological findings are individually identified in Appendix N.

7.12 Histopathology

Mortality occurred in one male rat at the 31.25 mg/kg-d dosage level but the extent of tissue autolysis at the time of necropsy complicated a thorough microscopic examination of harvested tissues and a cause of death could not be determined. Dark kidneys and dark spleens were noted throughout the male and female dose groups but did not have a correlating histologic finding. The dark organ appearance was likely due to blood congestion and was not considered treatment-related. Pale small intestines, typically in combination with the presence of yellow fluid, were also noted throughout all male and female dose groups. This observation was likely due to repeated oral corn oil administration and was confirmed microscopically, appearing as microvacuolation of the apical enterocytes.

In the liver, lymphocytic infiltration was noted in all male and female dose groups. Although the severity of this lesion may intensify with chemical exposures, a few isolated aggregates of

mononuclear cells are typically considered a background lesion (Thoolen, 2010). Lymphocytic infiltration (minimal to moderate) was observed in 9/10 males and 6/10 females in the control group, 9/10 males and 10/10 females at 31.25 mg/kg-d, 10/10 males and 8/10 females at 125 mg/kg-d, and 9/10 males and 6/10 females at 500 mg/kg-d. This observation was not considered treatment-related since incidence and severity was similar among all dose groups, including controls.

In the spleen, extramedullary hematopoesis and hemosiderosis was noted in the majority of male and female rats, including the females that did not become pregnant. Minimal to moderate extramedullary hematopoesis was observed in all male rats except for two in the 31.25 mg/kg-d group and in all female rats except for one control and one in the 500 mg/kg-d group. Minimal to moderate hematopoesis was noted in all male and female rats except for one male in the 31.25 mg/kg-d group. Both of these observations were not considered treatment-related since incidence and severity was similar among all dose groups, including controls.

A decrease in thymic cortical lymphocytes was noted in 5/8 controls, 3/9 31.25 mg/kg-d, 5/10 125 mg/kg-d, and 2/7 500 mg/kg-d pregnant females. In pregnant females, an early increase in thymic mass is followed by a marked reduction in cellularity of the cortex with the level of cellularity returning to normal once pregnancy is over. In addition, elevated progesterone levels during pregnancy negatively impact thymic mass whereas elevated prolactin levels during lactation have a stimulatory effect on the thymus (Pearse, 2006). Although this microscopic finding was observed in pregnant females in all dose groups, thymic mass was not different across dose groups. The finding was considered to be associated with pregnancy and not treatment-related.

NTO-related lesions were limited to the reproductive system in male rats. Severe tubular degeneration and atrophy was observed in the testes of 10/10 male rats given 500 mg/kg-d NTO. The majority of the testicular seminiferous tubules in these animals were shrunken, retaining only Sertoli cells, spermatogonia, and early stage spermatocytes (leptotene and zygotene). Few tubules retained pachytene spermatocytes and most were degenerate. One male control animal was noted to have a severely degenerative/atrophied left testis however the finding was considered to be a random congenital underdeveloped testis and not related to the study. Moderate hypospermia was observed in the epididymides of 3/10 500 mg/kg-d males while severe hypospermia/aspermia was observed in 7/10 500 mg/kg-d males. Moderate hypospermia was defined as absence of mature spermatids in the head and body of the epididymis with mature spermatids evident in the tail section. In animals with severe hypospermia/aspermia, mature spermatids were not observed in any epididymal segment. Minimal to mild cribiform change of the epididymis, defined as an infolding and bridging of the epithelium in segments of ducts that have undergone contraction due to decreased/absent sperm, was observed in 10/10 500 mg/kg-d male rats. Severe hypospermia/aspermia as well as cribiform change of the epididymis was also observed in the one male control animal with an underdeveloped testis.

Following a 4-week recovery period, complete recovery from observed testicular and epididymal lesions was not evident in male rats given 500 mg/kg-d NTO for 4 weeks. For recovery males, incidence and severity of testicular tubular degeneration/atrophy was reported separately for the left and right testes. Incidence and severity of tubular degeneration/atrophy for 500 mg/kg-d recovery males was as follows: 4/10 left testes and 4/8 right testes with mild degeneration, 4/10 left testes and 3/8 right testes with moderate degeneration, and 2/10 left testes and 1/8 right testes with severe degeneration. Two of the right testes in the 500 mg/kg-d recovery group were not available for microscopic evaluation. All spermatogonia and spermatocytes were present through all stages

for recovery males. Mild degeneration was defined as variable mature 13-18 and 19 spermatids missing while moderate degeneration indicated that spermatids 1-11/12 were generally present with some 7-10 spermatid loss and 13-18 variably present. No mature 19 spermatids were present with moderate degeneration. Severe degeneration was defined as stages I-V completely intact, stages VII-VIII missing mature 19 spermatids, and stages IX-XIV missing spermatids 9-14 with more completely atrophic tubules. Moderate hypospermia was observed in the epididymides of 7/9 500 mg/kg-d recovery males with 2/9 exhibiting severe hypospermia/aspermia. The testes and epididymides of all male control recovery animals were normal (see Appendix O).

8 Discussion

This study was conducted to provide additional information concerning the subacute (28 day) oral toxicity of NTO as well as provide preliminary reproductive/developmental toxicity information based on the results from previous studies. The previously performed subacute (14 day) and subchronic (90 day) oral toxicity studies identified the testes and epididymides as primary target organs for NTO-induced toxicity. In the subacute study, testes mass and mass ratios were reduced compared to controls in male rats administered 500 mg/kg-day NTO and above. Mean absolute epididymal mass was reduced in male rats given 1500 and 2000 mg/kg-d compared to controls. Histopathology was not performed on any tissues from the 14-day study. The subchronic study on NTO revealed reductions in both testes and epididymal mass and mass ratios at dosages of 315 mg/kg-day and above. Reductions in sperm counts were also noted at dosages of 315 mg/kg-day and above. Microscopic evaluation of the 90-day tissues revealed increased incidences of testicular hypoplasia, characterized by moderate to severe tubular degeneration/atrophy, at dosages of 315 mg/kg-day. Trace to mild hepatocellular hyperplasia was also observed in the livers of male and female rats administered 1000 mg/kg-d for 90 days (USAPHC (Provisional), 2010).

The results of the repeated dose toxicity portion of this study were generally in agreement with the previously reported subacute and subchronic studies. Dosages of NTO up to and including 500 mg/kg-d did not induce compound-related pre-term mortality and relevant clinical signs of toxicity were mainly limited to bright yellow-colored urine in the two highest dose groups. No differences between NTO-treated and control animals were observed for body mass, body mass change, food consumption, hematology, clinical chemistry, or prothrombin time.

NTO-induced toxicity was, once again, limited to the male reproductive organs following subacute (14- or 28-day) exposure. These test article-related lesions were characterized by severe testicular seminiferous tubule degeneration and moderate to severe hypospermia with cribiform change of the epididymides in 10/10 male 500 mg/kg-d rats. This spermatogenic disruption can be caused by a direct effect on the seminiferous epithelium, affecting either the Sertoli cell or any one of the germ cell populations, or may be a secondary response to altered hormone levels, altered vascular supply, or altered fluid balance within the testis or epididymis (Lanning et. al, 2002). Recent work has also shown that NTO does not appear to act directly as an anti-androgenic endocrine disrupting chemical (USAPHC, 2013). Since germ cells are entirely dependent on the coordinated functioning of all other cell types and processes within the testis, prolonged dosing with any testicular toxicant will typically result in germ cell degeneration or loss. In addition, each population of germ cells has its own sensitivity to different chemical toxicants. In repeated-dose studies of 4-13 weeks in duration using high dose levels, most testicular toxicants will produce a progressive germ cell loss (maturation depletion) resulting in tubules containing only Sertoli cells. Although also

sensitive to chemical damage, Sertoli and Leydig cells are extremely resistant to cell death and typically respond with biochemical disturbances. Both in this study as well as the previously-performed subchronic oral study with NTO, it was this germ cell maturation depletion that acted as a marker of injury but also reduced the specificity of the pattern of spermatogenic depletion. Since the pattern of disturbance is typically only seen during the early development of the lesion, it would be necessary to conduct a time course study to identify the earliest pathological changes (Creasy, 1997).

Following a 4-week recovery period, microscopic evaluation of the testes of the satellite 500 mg/kg-d male rats revealed that they had not completely recovered. Although all spermatogonia and spermatocytes were present through all of the stages, maturing spermatids were only variably present. Cauda epididymal sperm counts in the recovery 500 mg/kg-d males recovered slightly to 28.6% of recovery control counts, compared to 6.8% for the 500 mg/kg-d main study males, but no motile sperm were detected. It is possible that the 4-week duration of the recovery period may have prevented a more complete recovery in these animals. It has been reported that recovery studies should be timed as multiples of the spermatogenic process (8 weeks for rats) to allow for any reversible injury to the spermatogonia to work its way through maturation depletion and allow full recovery of all cell layers. In addition, there is often a lag period before all the cells reach full production capacity so it is possible that recovery may take even longer than 1 full period. In theory, if spermatogonia are still present then the lesion is potentially reversible but if the Sertoli cells are functionally compromised, spermatogenesis may not be supportable. Conversely, spermatogonia may be significantly depleted, but if the Sertoli cells are functionally intact and sufficient time is allowed for stem cell renewal and repopulation, substantial recovery may be seen (Creasy, 1997 and Lanning et. al., 2002). The lack of a defined mechanism for germ cell loss in this study as well as the length of the recovery period prevented a prediction of recovery from NTO-induced testicular injury.

The design of this study was based closely on the procedures outlined in OECD Health Effects Test Guideline 422 for a combined repeated-dose toxicity test with the reproduction/developmental screening test (OECD, 1996). These procedures included a 2-week pre-mating/dosing period prior to pair-housing for mating. The results of this study did not show any reduction in the number of females becoming pregnant between NTO-treated and control animals. Based upon the microscopic evaluation of the 500 mg/kg-d males tissues, revealing severe tubular degeneration/atrophy, and the sperm analysis, indicating a severe reduction in sperm count and no motile sperm, very few of the 500 mg/kg-d female rats should have become pregnant during the mating period. Although it is speculation at this point, the results observed were most likely a function of the pre-mating period duration. Had the pre-mating period been extended to a 6-8 week duration, the male gonadal regression observed would likely have progressed enough to result in male infertility at 500 mg/kg-d. Even though the spermatogenic cycle repeats approximately every 12.9 days in the Sprague-Dawley rat, the complete process of spermatogenesis requires approximately 56 days or 4.5 cycles (Creasy, 1997). The elongating spermatids shed during a previously unaffected spermatogenic cycle likely allowed for fertility to be observed in the male 500 mg/kg-d animals. None of the other reproductive/litter parameters evaluated for NTO-treated animals during this study exhibited differences compared to control animals or historical averages. By guideline, monitoring for developmental effects on the offspring only occurred on the day of birth (day 0) and the day of scheduled euthanasia (day 4) and was limited to a gross external examination. Both pre- and post-natal loss was highest in the corn oil control group compared to NTO-treated animals. Several litters throughout most of the dose groups, including controls, had to

be humanely euthanized due to total neglect/abandonment by the dam. All of the pups euthanized pre-term were included as post-natal deaths and only one dam in the 125 mg/kg-d group exhibited gross and microscopic findings upon necropsy that may have contributed to the abandonment. It is undetermined if treatment with NTO contributed to the dam's overall compromised and ill state. No gross abnormalities or abnormal behavior was observed in any of the pups at either observation time.

9 Conclusions

Daily oral exposure to male and female rats at dosages of 31.25, 125, and 500 mg/kg-d NTO in corn oil for four weeks did not induce compound-related pre-term mortality. Clinical signs of toxicity were mainly limited to bright yellow-colored urine at higher dosages with no changes in body mass, body mass gain, and food consumption compared to controls observed throughout the study period.

Treatment with NTO resulted in reductions in testes and epididymides mass and mass ratios in male rats given 500 mg/kg-d. Microscopic evaluation of these tissues revealed severe degeneration/atrophy of the testicular seminiferous tubules along with moderate to severe hypospermia and cribiform change of the epididymides. Sperm counts were reduced in the high dose group (500 mg/kg-d) with no motile sperm observed. Complete recovery was not evident in the high dose satellite group following a 4-week recovery period. Reductions in sperm counts with no motile sperm were also observed in the male satellite group. In female rats, differences between NTO treated and control animals were observed for brain and spleen, and uterus mass and mass ratios. Absolute brain mass was reduced in the 31.25 mg/kg-d dose group relative to controls. Spleen mass and spleen to body mass ratios were reduced in the 31.25 and 500 mg/kg-d dose groups relative to controls. No treatment-related microscopic findings were observed in female rats.

Under the stated study conditions, oral dosages of up to and including 500 mg/kg-d NTO did not appear to affect reproduction or development in Sprague-Dawley rats. Based upon the gross and microscopic findings in male rats following 4 weeks of treatment, as well as the results of the cauda epididymal sperm analysis, infertility would have likely been observed in the high dose males with an extended pre-mating dosing period. Gross external examinations of the offspring on the day of birth and on day 4 postpartum did not indicate that NTO presents a developmental hazard.

10 Point of Contact

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Appendix A

References

BAE Systems Ordnance Systems, Inc. 2007. Material Safety Data Sheet (MSDS) on NTO. Prepared by BAE Systems Ordnance Systems, Inc., 4509 West Stone Drive, Kingsport, TN 37660-9982.

Charles River Laboratories. 1993. Historical Control Data for Development and Reproductive Toxicity Studies using the Crl:CD BR Rat. Compiled by the Middle Atlantic Reproduction and Teratology Association.

Charles River Laboratories. 2006. Clinical Laboratory Parameters for Crl:CD(SD) Rats. Prepared by M.L.A. Giknis, Ph.D. and C.B. Clifford, D.V.M., Ph.D.

Creasy, D.M. 1997. Evaluation of Testicular Toxicity in Safety Evaluation Studies: The Appropriate Use of Spermatogenic Staging. *Toxicol. Pathol.*, 25(2): 119-131.

Davies, P.J. and A. Provatas. 2006. Characterization of 2,4-Dinitroanisole: An Ingredient for Use in Low Sensitivity Melt Cast Formulations. *Defense Science and Technology Organization, Edinburgh South Australia.*

Department of the Army. 2003. Regulation 70-1, *Army Acquisition Policy*. http://www.apd.army.mil/pdffiles/r70_1.pdf.

Department of the Army. 2007a. Regulation 200-1, *Environmental Protection and Enhancement*. http://www.apd.army.mil/pdffiles/r200_1.pdf.

Department of the Army. 2007b. Regulation 40-5, *Preventive Medicine*. http://www.apd.army.mil/pdffiles/r40_5.pdf.

Department of the Army. 2008 (Rapid Action Revision 2009). Pamphlet 70-3, *Army Acquisition Procedures for Insensitive Munitions/Unplanned Stimuli*. http://www.apd.army.mil/pdffiles/p70_3.pdf.

Department of Defense. 1996. Department of Defense Instruction 4715.4, *Pollution Prevention*. http://www.dtic.mil/whs/directives/corres/pdf/471504p.pdf.

Duncan, K. 2002. Insensitive Munitions and the Army: Improving Safety and Survivability. *Army Logistician PB700-02-1* Volume 34, Issue 1: 16-17.

Lanning, L.L., Creasy, D.M., Chapin, R.E., Mann, P.C., Barlow, N.J., Regan, K.S., Goodman, D.G. 2002. Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity. *Toxicol. Pathol.*, 30(4): 507-520.

Los Alamos National Laboratory. 1985. Report No. LA-10533-MS. *A, Toxicological Study of NTO*. Los Alamos National Laboratory, Los Alamos, New Mexico. Prepared by J.E. London and D.M. Smith.

Organization for Economic Cooperation and Development. 1996. OECD Guideline for Testing of

Chemicals: OECD 422. *Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening* Test.

Pearse, Gail. 2006. Histopathology of the Thymus. *Toxicol. Pathol.*, 34: 515-547.

Smith, M.W. and M.D. Cliff. 1999. NTO-Based Explosive Formulations: A Technology Review. Weapons Systems Division, DSTO Aeronautical and Maritime Research Laboratory, Salisbury South Australia 5108 Australia, DSTO-TR-0796.

Spear, R.J., Louey, C.N. and M.G. Wolfson. 1989. A Preliminary Assessment of NTO as an Insensitive High Explosive. *DSTO Materials Research Laboratory, Maribyrnong, Victoria 3032, Australia, MRL-TR-89-18*.

Thoolen B., Maronpot R.R., Harada T., Nyska A., Rousseaux C., Nolte T., Malarkey D.E., Kaufmann W., Kuttler K., Deschl U., Nakae D., Gregson R., Vinlove M.P., Bix A.E., Singh B., Belpoggi F., Ward J.M. 2010. Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System. *Toxicol Pathol.*, 38:5S-81S.

U.S. Army Environmental Command (USAEC). 2009. Final Report. *FY09 Army Environmental Requirements and Technology Assessments (AERTA)*. Aberdeen Proving Ground Maryland.

U.S. Army Public Health Command. 2013. Toxicology Report No. 87-XE-0FV5-12. *Assessment of 3-Nitro-1,2,4-Triazol-5-One (NTO) as a Potential Endocrine Disrupting Chemical in Rats Using the Hershberger and Uterotrophic Bioassays*. Aberdeen Proving Ground, Maryland.

U.S. Army Public Health Command (Provisional). 2010. Toxicology Study No. 85-XC-0A6W-08. *Subchronic Oral Toxicity of NTO in Rats*. Aberdeen Proving Ground, Maryland.

APPENDIX B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. 85-XC-0FP4-12, Protocol No. 0FP4-93-12-03-03, *"Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat, April – July 2012"*, the following critical phases were audited by the Quality Systems Office:

PRE IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Study Protocol Good Laboratory Practice Standards and Animal Care Review	01/19/2012	01/19/2012

IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Test System - Husbandry, Body Weights and Food Consumption,	05/18/2012	05/31/2012
Test System - Identification and Observations	05/18/2012	05/31/2012
Administration Procedures of the Test Substance by Oral Gavage Dose	05/18/2012	05/31/2012
Compliance with Study Protocol Modification	05/24/2012	05/31/2012
Study Personnel Training Records	05/29/2012	06/08/2012
Co-Housing Period - Procedures, Observations and Raw Data Documentation	05/31/2012	06/07/2012
Co-Housing Period - Special Husbandry Procedures	05/31/2012	06/07/2012
Male Rats - Study Endpoint, Anesthesia, Euthanasia, Necropsy and Tissue Collection Procedures	06/05/2012	06/13/2012
Sperm Extrusion and Viability and Motility Analysis Procedures	06/05/2012	06/13/2012
Analytical Chemistry Support - Dosing Solution Concentration Verification	06/12/2012	06/18/2012
Raw Data Documentation Procedures	07/02/2012	07/06/2012
Study Final Animal In-Life Endpoint Criteria	07/02/2012	07/06/2012

POST IN-LIFE PHASE OF THE STUDY

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Pathology Contributing Scientist Report Review	07/09/2013	07/09/2013

APPENDIX B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. 85-XC-0FP4-12, Protocol No. 0FP4-93-12-03-03, *"Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat, April – July 2012"*, the following critical phases were audited by the Quality Systems Office:

POST IN-LIFE PHASE OF THE STUDY (Continued)

Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Final Study Report Review	10/17/2013	10/18/2013
Study Raw Data Review	10/17/2013	10/18/2013

Note 1 All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Note 2 In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.

Note 3 This report has been audited by the Quality Assurance Unit (QSO), and is considered to be an accurate account of the data generated and of the procedures followed

Michael P. Kefauver — 21 March 2014

Michael P. Kefauver
Quality Assurance Specialist, QSO

Date

Appendix C

Archives and Study Personnel

1. ARCHIVES.

a. All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, Building E-2100, USAPHC, for a minimum of five (5) years following submission of the final report to the Sponsor.

b. Records on animal receipt, diet, and facility environmental parameters will be archived by the Veterinary Medical Division, Toxicology Portfolio, for a minimum of five (5) years following submission of the final report to the Sponsor.

c. Some ancillary records pertaining to this study, such as instrument maintenance logs, animal room observation logs, etc., will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, Building E-2100, USAPHC.

d. Wet tissues, histology slides, and paraffin blocks are stored in building E-5158.

2. PERSONNEL.

a. Management

(1) Management (In-Life): COL Chris E. Hanson, Portfolio Director, Toxicology; Glenn J. Leach, Ph.D., Program Manager, Toxicity Evaluation Program (TEP); Dr. Mark S. Johnson, Ph.D., Program Manager, Health Effects Research Program (HERP).

(2) Management (Report): Mark S. Johnson, Portfolio Director, Toxicology; Arthur J. O'Neill, Program Manager, TEP; Dr. Michael Quinn, Ph.D., Program Manager, HERP.

b. Study Director: Lee C.B. Crouse, Biologist, TEP.

c. Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems Office.

d. Veterinary Support and Animal Care: Dawn C. Fitzhugh, DVM, LTC, VC; Robert Sunderland, Animal Health Technician; Rebecca Kilby, Animal Health Technician; Jason Williams, Animal Health Technician.

e. Pathology Lab Coordinator: Patricia A. Beall, Biologist, TEP.

f. Histopathology: Shannon M. Wallace, DVM, DACVP, LTC, VC, Pathologist, VMD.

g. In-Life Support: Emily May Lent, Toxicologist, TEP.

h. Hematology, Clinical Chemistry, Urinalysis: Matthew A. Bazar, Biologist, TEP; Mark R. Way, Biologist, TEP.

i. Archivist: Martha L. Thompson, Data Acquisition Specialist, TEP.

Appendix D

Clinical Observations

Table D-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Daily Observations
Male Rats

Group	Animal ID	Observation	First Day* Observed	Last Day* Observed
Corn Oil Control	12-0163	None		
	12-0205	Barbering left front limb	25	28
	12-0206	None		
	12-0207	Animal crumbling food on bottom of cage; Suspect food consumption	6	6
	12-0235	None		
	12-0254	None		
	12-0263	None		
	12-0267	Chromodacryorrhea right eye	22	22
	12-0284	None		
	12-0313	None		
31.25 mg/kg	12-0164	None		
	12-0165	Small sore on right side of mouth	5	6
		Dosing material on chin	17	17
	12-0225	None		
	12-0234	None		
	12-0243	None		
	12-0245	None		
	12-0255	Barbering both front limbs	13	28
	12-0262	None		
	12-0324	Dried red material around nose	16	21
	12-0351	Found dead	18	18
125 mg/kg	12-0187	None		
	12-0204	Dosing material on chin	2	2
	12-0223	Dosing material on chin	0	27
	12-0224	None		
	12-0253	None		
	12-0275	Dosing material on chin; Likely aspirated	1	1
		Barbering both front limbs	6	28
	12-0293	Barbering both front limbs	8	28
	12-0296	Dosing material on chin	1	1
	12-0345	None		
	12-0354	Bright yellow stained bedding	0	0
500 mg/kg	12-0177	None		
	12-0189	None		
	12-0203	None		
	12-0209	None		
	12-0236	Dosing material on chin	1	9
		Congested breathing	15	15
	12-0256	Dosing material on chin	8	10
		Slight congested breathing	14	14
	12-0297	None		
	12-0314	Dosing material on chin	20	20
	12-0322	Dosing material on chin	1	1
		Dried red material around nose	21	22
		Small cut below right nostril	21	22
		Barbering both front limbs	25	28
	12-0341	None		

* = Signs may be observed intermittently between first and last day.

Table D-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Daily Observations
Female Rats

Group	Animal ID	Observation	First Day* Observed	Last Day* Observed
Corn Oil Control	12-0211	Barbering both front limbs	35	44
	12-0220	None		
	12-0222	None		
	12-0260	Barbering both front limbs	31	42
	12-0289	Animal inadvertently given 1.15 ml of 6.25 mg/ml NTO suspension	4	4
	12-0298[a]	Small scab behind left ear	41	42
	12-0306	Barbering both front limbs	22	42
	12-0309	None		
	12-0317[b]	Chromodacryorrhea right eye	19	23
		Chromodacryorrhea both eyes	24	24
	12-0339	Barbering right front limb	43	44
31.25 mg/kg	12-0168	Dosing material on chin	5	33
	12-0170	None		
	12-0201[c]	Dosing material on chin	8	16
	12-0221	Dried red material around nose and on both front limbs and head	49	49
		Dried red material around urogenital area	50	50
	12-0240	Barbering both front limbs	30	42
	12-0299[a]	Barbering both front limbs	35	45
	12-0300	Dosing material on chin	22	22
	12-0346	None		
	12-0367	Dried red material around nose	37	37
	12-0369	None		
125 mg/kg	12-0169	Dosing material on chin	1	5
		Barbering both front limbs	27	45
		Blood and bright yellow staining of urogenital area	40	41
	12-0210	Dosing material on chin	5	8
	12-0212	None		
	12-0227	Blood still visible in vaginal area on postpartum day 1	39	39
	12-0257	Barbering right front limb	18	18
		Barbering both front limbs	19	44
	12-0259	Animal inadvertently given 1.03 ml of 100 mg/ml NTO suspension	9	9
		Barbering both front limbs	23	45
		Blood and bright yellow staining of urogenital area	41	41
		Rough haircoat	44	45
	12-0261	None		
	12-0278	Dosing material on chin	2	2
	12-0291	Sores on right below ear and on left dorsal cervical area	6	45
		Blood and bright yellow staining of urogenital area	41	42
	12-0327	Barbering both front limbs	27	45
500 mg/kg	12-0166	None		
	12-0167	None		
	12-0171[a]	Bright yellow stained bedding	0	0
		Kicked during dosing causing blood around mouth	7	7
		Congested breathing	7	10
		Dosing material on chin	12	41
	12-0198	None		
	12-0229[a]	Dosing material on chin	0	0
	12-0258	Dosing material on chin	1	1
		Barbering both front limbs	29	34
		Dried red material around nose and on both front limbs	36	36
		Blood and bright yellow urine staining of urogenital area	37	38
		Soft stool	38	38
	12-0279	Barbering right front limb	24	28
		Barbering both front limbs	29	42
		Congested breathing	30	36
		Blood and bright yellow urine staining of urogenital area	37	37
	12-0308[c]	None		
	12-0336	Dosing material on chin	0	0
		Congested breathing	10	28
		Barbering both front limbs	39	42
	12-0348	Bright yellow stained bedding	0	0

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.
* = Signs may be observed intermittently between first and last day

Table D-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Daily Observations
Recovery Male Rats

Group	Animal ID	Observation	First Day* Observed	Last Day* Observed
Corn Oil Control	12-0174	Small abrasion with scab on right dorsal cervical region	14	15
		Small area of hair loss on right dorsal cervical region	16	17
	12-0179	None		
	12-0194	None		
	12-0208	None		
	12-0272	None		
	12-0294	None		
	12-0304	Chromodacryorrhea right eye	16	16
		Barbering left front limb	19	21
		Barbering both front limbs	22	36
	12-0323	Dried red material around left eye	50	57
	12-0332	None		
	12-0365	None		
500 mg/kg	12-0176	Bright yellow-stained bedding	0	0
	12-0186	None		
	12-0215	Scab behind left ear	29	57
		Abrasions behind both ears	36	43
		Abrasion behind left ear	50	50
	12-0244	None		
	12-0283	None		
	12-0295	Bright yellow-stained bedding	0	0
		Dosing material on chin	22	22
	12-0302	None		
	12-0310	Dosing material on chin	0	0
		Barbering both front limbs	36	57
	12-0333	None		
	12-0363	Bright yellow-stained bedding	0	0

* = Signs may be observed intermittently between first and last day.

Appendix E

Individual and Summary of Body Mass Data

Table E-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Body Mass (grams)
Male Rats

Group	Animal ID	Day 0	Day 7	Day 14	Day 21	Day 27*	Day 28**
Corn Oil Control	12-0163	337	397	448	468	499	486
	12-0205	327	379	422	451	489	464
	12-0206	304	363	415	453	492	473
	12-0207	320	379	432	470	512	483
	12-0235	378	446	507	544	581	572
	12-0254	323	377	425	461	492	483
	12-0263	333	391	441	476	517	507
	12-0267	361	424	478	521	558	540
	12-0284	364	419	481	511	541	515
	12-0313	349	402	458	484	517	500
	Mean	**339.6**	**397.7**	**450.7**	**483.9**	**519.8**	**502.3**
	SD	**22.98**	**25.55**	**29.99**	**31.26**	**31.02**	**33.01**
31.25 mg/kg	12-0164	314	369	418	444	465	452
	12-0165	324	378	425	462	501	488
	12-0225	332	395	447	472	505	493
	12-0234	318	365	400	430	458	440
	12-0243	326	375	418	424	455	441
	12-0245	341	387	440	472	504	477
	12-0255	312	352	391	420	437	425
	12-0262	363	431	487	527	576	557
	12-0324	364	432	482	508	552	526
	12-0351	372	438	493	(f)	(f)	(f)
	Mean	**336.6**	**392.2**	**440.1**	**462.1**	**494.8**	**477.7**
	SD	**22.30**	**30.94**	**36.55**	**37.24**	**46.45**	**43.54**
125 mg/kg	12-0187	352	408	455	493	533	517
	12-0204	335	376	417	457	496	478
	12-0223	342	400	451	481	521	500
	12-0224	356	412	436	470	513	488
	12-0253	300	349	391	419	446	426
	12-0275	348	392	435	463	488	472
	12-0293	336	394	440	467	484	473
	12-0296	320	367	407	427	457	447
	12-0345	326	375	418	436	467	444
	12-0354	367	416	459	484	514	494
	Mean	**338.2**	**388.9**	**430.9**	**459.7**	**491.9**	**473.9**
	SD	**18.44**	**20.55**	**21.03**	**23.70**	**27.40**	**26.60**
500 mg/kg	12-0177	353	421	483	525	566	553
	12-0189	369	409	456	480	524	505
	12-0203	321	373	423	450	488	465
	12-0209	344	398	460	494	537	513
	12-0236	350	413	449	494	536	508
	12-0256	320	362	378	395	430	411
	12-0297	364	430	455	493	531	515
	12-0314	380	418	454	469	503	486
	12-0322	300	339	380	377	394	382
	12-0341	348	380	412	447	476	448
	Mean	**344.9**	**394.3**	**435.0**	**462.4**	**498.5**	**478.6**
	SD	**24.69**	**29.60**	**35.39**	**46.47**	**53.13**	**52.37**

* Pre-fasted final body weights.
** Fasted final body weights
(f) = Animal died on study.

Table E-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Pre-Pregnancy Individual Body Mass (grams)
Female Rats

Group	Animal ID	Day 0	Day 7	Day 14	Day 21	Day 28	Day 34	Day 41
Corn Oil Control	12-0211	224	245	264				
	12-0220	223	243	263				
	12-0222	216	222	251				
	12-0260	218	215	233				
	12-0289	194	215	233				
	12-0298[a]	199	214	230				
	12-0306	204	222	237				
	12-0309	230	235	248				
	12-0317[b]	238	253	264	315	336	331	342
	12-0339	195	208	224				
	Mean	**214.1**	**227.2**	**244.7**	**315.0**	**336.0**	**331.0**	**342.0**
	SD	**15.34**	**15.59**	**15.30**				
31.25 mg/kg	12-0168	226	250	264				
	12-0170	217	241	264				
	12-0201[c]	228	238	272	295			
	12-0221	227	242	260	280			
	12-0240	200	225	237				
	12-0299[a]	230	255	272				
	12-0300	222	230	252				
	12-0346	200	210	229				
	12-0367	202	228	247				
	12-0369	215	238	252				
	Mean	**216.7**	**235.7**	**254.9**	**287.5**			
	SD	**12.03**	**13.00**	**14.33**	**10.61**			
125 mg/kg	12-0169	213	231	244				
	12-0210	224	240	247				
	12-0212	213	229	245				
	12-0227	222	244	265				
	12-0257	204	202	215				
	12-0259	207	224	241				
	12-0261	212	215	228				
	12-0278	238	246	258				
	12-0291	212	236	235				
	12-0327	223	241	261				
	Mean	**216.8**	**230.8**	**243.9**				
	SD	**9.50**	**13.23**	**14.57**				
500 mg/kg	12-0166	211	228	245				
	12-0167	220	239	245	267			
	12-0171[a]	228	230	247				
	12-0198	211	237	260				
	12-0229[a]	201	206	224				
	12-0258	216	214	228				
	12-0279	221	220	239				
	12-0308[c]	229	249	255	300			
	12-0336	180	193	190				
	12-0348	227	230	242				
	Mean	**214.4**	**224.6**	**237.5**	**283.5**			
	SD	**14.98**	**16.68**	**19.91**	**23.33**			

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.

Table E-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Gestational and Postpartum Individual Body Mass (grams)
Female Rats

		Gestational Days				Postpartum Days	
Group	Animal ID	Day 0	Day 7	Day 14	Day 20	Day 0	Day 4
	12-0211	265	306	348	410	365	356
Corn Oil	12-0220	272	317	365	442	313	333
Control	12-0222	256	300	345	424	335	341
	12-0260	242	289	344	415	275	ND
	12-0289	231	290	330	410	290	304
	12-0298[a]						
	12-0306	242	282	325	399	283	270
	12-0309	251	295	338	413	295	313
	12-0317[b]						
	12-0339	231	269	308	383	246	267
	Mean	**248.8**	**293.5**	**337.9**	**412.0**	**300.3**	**312.0**
	SD	**15.04**	**14.73**	**17.10**	**17.20**	**36.98**	**34.33**
	12-0168	268	320	367	440	329	ND
31.25 mg/kg	12-0170	266	322	375	480	334	342
	12-0201[c]					328	335
	12-0221	293	344	389	453	305	343
	12-0240	243	279	323	385	314	ND
	12-0299[a]						
	12-0300	256	288	318	387	292	319
	12-0346	224	257	296	363	265	296
	12-0367	254	294	330	397	292	283
	12-0369	262	303	341	421	297	311
	Mean	**258.3**	**300.9**	**342.4**	**415.8**	**306.2**	**318.4**
	SD	**20.02**	**27.50**	**31.89**	**39.71**	**22.40**	**23.27**
	12-0169	255	309	354	427	266	315
125 mg/kg	12-0210	262	313	352	407	363	364
	12-0212	250	297	341	412	323	325
	12-0227	272	322	368	435	335	335
	12-0257	232	280	319	393	284	311
	12-0259	250	302	340	405	273	243
	12-0261	245	295	346	421	303	336
	12-0278	272	333	388	493	379	370
	12-0291	252	285	334	400	273	294
	12-0327	263	303	340	408	319	313
	Mean	**255.3**	**303.9**	**348.2**	**420.1**	**311.8**	**320.6**
	SD	**11.74**	**15.31**	**18.08**	**27.08**	**37.16**	**34.20**
	12-0166	263	304	299	383	316	321
500 mg/kg	12-0167	270	310	345	417	320	343
	12-0171[a]						
	12-0198	264	315	353	439	326	351
	12-0229[a]						
	12-0258	230	287	336	399	277	312
	12-0279	248	287	338	372	264	274
	12-0308[c]					336	352
	12-0336	192	236	282	350	255	ND
	12-0348	236	277	329	419	322	320
	Mean	**243.3**	**288.0**	**326.0**	**397.0**	**302.0**	**324.7**
	SD	**27.12**	**26.76**	**25.85**	**30.77**	**31.46**	**27.51**

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.
ND = No data

Table E-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Body Mass (grams)
Recovery Male Rats

		Dosing Period					Recovery Period				
Group	Animal ID	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 55*	Day 56**
Corn Oil Control	12-0174	329	386	446	499	546	579	600	620	635	618
	12-0179	343	418	472	530	582	610	623	655	673	653
	12-0194	385	439	482	536	595	627	640	670	685	663
	12-0208	319	381	434	474	525	546	572	593	610	595
	12-0272	355	414	465	508	552	567	584	613	635	617
	12-0294	339	393	452	487	529	550	575	603	611	588
	12-0304	367	423	474	512	555	571	602	625	642	625
	12-0323	304	361	410	454	493	526	556	585	592	572
	12-0332	288	329	366	396	431	446	462	482	500	482
	12-0365	347	400	450	480	502	528	559	567	580	558
	Mean	**337.6**	**394.4**	**445.1**	**487.6**	**531.0**	**555.0**	**577.3**	**601.3**	**616.3**	**597.1**
	SD	**28.95**	**32.36**	**34.96**	**40.89**	**47.45**	**50.27**	**48.66**	**52.07**	**52.49**	**52.25**
500 mg/kg	12-0176	371	426	477	517	562	588	602	621	632	611
	12-0186	357	428	476	518	556	568	595	616	621	599
	12-0215	349	407	461	513	552	570	581	619	636	614
	12-0244	310	359	405	439	469	493	502	518	524	510
	12-0283	290	333	371	402	439	460	486	509	523	510
	12-0295	335	393	443	484	513	536	572	600	619	602
	12-0302	379	426	482	525	564	603	636	666	684	664
	12-0310	357	400	450	484	528	566	598	617	644	621
	12-0333	298	342	380	422	462	473	505	529	554	528
	12-0363	334	393	430	465	501	527	548	561	582	561
	Mean	**338.0**	**390.7**	**437.5**	**476.9**	**514.6**	**538.4**	**562.5**	**585.6**	**601.9**	**582.0**
	SD	**30.42**	**34.88**	**40.28**	**43.74**	**45.62**	**49.32**	**50.27**	**52.91**	**53.98**	**52.20**

* Pre-fasted final body weights.
** Fasted final body weights
(f) = Animal died on study.

Table E-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Summary of Body Mass (grams)
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Day 0	Mean	339.6	336.6	338.2	344.9
	S.D.	22.98	22.30	18.44	24.69
	N	10	10	10	10
Day 7	Mean	397.7	392.2	388.9	394.3
	S.D.	25.55	30.94	20.55	29.60
	N	10	10	10	10
Day 14	Mean	450.7	440.1	430.9	435.0
	S.D.	29.99	36.55	21.03	35.39
	N	10	10	10	10
Day 21	Mean	483.9	462.1	459.7	462.4
	S.D.	31.26	37.24	23.70	46.47
	N	10	9	10	10
Day 27*	Mean	519.8	494.8	491.9	498.5
	S.D.	31.02	46.45	27.40	53.13
	N	10	9	10	10
Day 28**	Mean	502.3	477.7	473.9	478.6
	S.D.	33.01	43.54	26.60	52.37
	N	10	9	10	10

* Pre-fasted final body weights.
** Fasted final body weights

Table E-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Pre-Pregnancy Body Mass (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil		
			31.25 mg/kg	125 mg/kg	500 mg/kg
Day 0	Mean	214.1	216.7	216.8	214.4
	S.D.	15.34	12.03	9.5	14.98
	N	10	10	10	10
Day 7	Mean	227.2	235.7	230.8	224.6
	S.D.	15.59	13	13.23	16.68
	N	10	10	10	10
Day 14	Mean	244.7	254.9	243.9	237.5
	S.D.	15.30	14.33	14.57	19.91
	N	10	10	10	10

Table E-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Gestational and Postpartum Body Mass (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Gestational Days					
Day 0	Mean	248.8	258.3	255.3	243.3
	S.D.	15.04	20.02	11.74	27.12
	N	8	8	10	7
Day 7	Mean	293.5	300.9	303.9	288.0
	S.D.	14.73	27.50	15.31	26.76
	N	8	8	10	7
Day 14	Mean	337.9	342.4	348.2	326.0
	S.D.	17.10	31.89	18.08	25.85
	N	8	8	10	7
Day 20	Mean	412.0	415.8	420.1	397.0
	S.D.	17.20	39.71	27.08	30.77
	N	8	8	10	7
Postpartum Days					
Day 0	Mean	300.3	306.2	311.8	302.0
	S.D.	36.98	22.40	37.16	31.46
	N	8	9	10	8
Day 4	Mean	312.0	318.4	320.6	324.7
	S.D.	34.33	23.27	34.20	27.51
	N	7	7	10	7

Table E-8
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Summary of Body Mass (grams)
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Day 0	Mean	337.6	338.0
	S.D.	28.95	30.42
	N	10	10
Day 7	Mean	394.4	390.7
	S.D.	32.36	34.88
	N	10	10
Day 14	Mean	445.1	437.5
	S.D.	34.96	40.28
	N	10	10
Day 21	Mean	487.6	476.9
	S.D.	40.89	43.74
	N	10	10
Day 28	Mean	531.0	514.6
	S.D.	47.45	45.62
	N	10	10
Day 35	Mean	555.0	538.4
	S.D.	50.27	49.32
	N	10	10
Day 42	Mean	577.3	562.5
	S.D.	48.66	50.27
	N	10	10
Day 49	Mean	601.3	585.6
	S.D.	52.07	52.91
	N	10	10
Day 55*	Mean	616.3	601.9
	S.D.	52.49	53.98
	N	10	10
Day 56**	Mean	597.1	582.0
	S.D.	52.25	52.20
	N	10	10

* = Unfasted final body weight.
* = Fasted final body weight.

Appendix F

Individual and Summary of Body Mass Gain Data

Table F-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Body Mass Gains (grams)
Male Rats

Group	Animal ID	Days 0-7	Days 7-14	Days 14-21	Days 21-27
Control	12-0163	60	51	20	31
	12-0205	52	43	29	38
	12-0206	59	52	38	39
	12-0207	59	53	38	42
	12-0235	68	61	37	37
	12-0254	54	48	36	31
	12-0263	58	50	35	41
	12-0267	63	54	43	37
	12-0284	55	62	30	30
	12-0313	53	56	26	33
	Mean	**58.1**	**53.0**	**33.2**	**35.9**
	S.D.	**4.91**	**5.72**	**6.84**	**4.36**
31.25 mg/kg	12-0164	55	49	26	21
	12-0165	54	47	37	39
	12-0225	63	52	25	33
	12-0234	47	35	30	28
	12-0243	49	43	6	31
	12-0245	46	53	32	32
	12-0255	40	39	29	17
	12-0262	68	56	40	49
	12-0324	68	50	26	44
	12-0351	66	55	(f)	(f)
	Mean	**55.6**	**47.9**	**27.9**	**32.7**
	S.D.	**10.15**	**6.95**	**9.66**	**10.26**
125 mg/kg	12-0187	56	47	38	40
	12-0204	41	41	40	39
	12-0223	58	51	30	40
	12-0224	56	24	34	43
	12-0253	49	42	28	27
	12-0275	44	43	28	25
	12-0293	58	46	27	17
	12-0296	47	40	20	30
	12-0345	49	43	18	31
	12-0354	49	43	25	30
	Mean	**50.7**	**42.0**	**28.8**	**32.2**
	S.D.	**6.00**	**7.10**	**7.08**	**8.20**
500 mg/kg	12-0177	68	62	42	41
	12-0189	40	47	24	44
	12-0203	52	50	27	38
	12-0209	54	62	34	43
	12-0236	63	36	45	42
	12-0256	42	16	17	35
	12-0297	66	25	38	38
	12-0314	38	36	15	34
	12-0322	39	41	-3	17
	12-0341	32	32	35	29
	Mean	**49.4**	**40.7**	**27.4**	**36.1**
	S.D.	**12.99**	**14.94**	**14.66**	**8.14**

(f) = Animal died on study.

Table F-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Pre-Pregnancy Individual Body Mass Gains (grams)
Female Rats

Group	Animal ID	Days 0-7	Days 7-14
Corn Oil Control	12-0211	21	19
	12-0220	20	20
	12-0222	6	29
	12-0260	-3	18
	12-0289	21	18
	12-0298	15	16
	12-0306	18	15
	12-0309	5	13
	12-0317	15	11
	12-0339	13	16
	Mean	**13.1**	**17.5**
	SD	**8.02**	**4.88**
31.25 mg/kg	12-0168	24	14
	12-0170	24	23
	12-0201	10	34
	12-0221	15	18
	12-0240	25	12
	12-0299	25	17
	12-0300	8	22
	12-0346	10	19
	12-0367	26	19
	12-0369	23	14
	Mean	**19.0**	**19.2**
	SD	**7.35**	**6.27**
125 mg/kg	12-0169	18	13
	12-0210	16	7
	12-0212	16	16
	12-0227	22	21
	12-0257	-2	13
	12-0259	17	17
	12-0261	3	13
	12-0278	8	12
	12-0291	24	-1
	12-0327	18	20
	Mean	**14.0**	**13.1**
	SD	**7.91**	**6.09**
500 mg/kg	12-0166	17	17
	12-0167	19	6
	12-0171	2	17
	12-0198	26	23
	12-0229	5	18
	12-0258	-2	14
	12-0279	-1	19
	12-0308	20	6
	12-0336	13	-3
	12-0348	3	12
	Mean	**10.2**	**12.9**
	SD	**9.99**	**7.81**

Table F-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Gestational and Postpartum Individual Body Mass Gains (grams)
Female Rats

		Gestational Days				Postpartum Days
Group	Animal ID	Days 0-7	Days 7-14	Days 14-20	Days 20 - PP Day 0	Days 0-4
	12-0211	41	42	62	-45	-9
Corn Oil	12-0220	45	48	77	-129	20
Control	12-0222	44	45	79	-89	6
	12-0260	47	55	71	-140	ND
	12-0289	59	40	80	-120	14
	12-0298[a]					
	12-0306	40	43	74	-116	-13
	12-0309	44	43	75	-118	18
	12-0317[b]					
	12-0339	38	39	75	-137	21
	Mean	**44.8**	**44.4**	**74.1**	**-111.8**	**8.1**
	SD	**6.45**	**5.13**	**5.67**	**31.24**	**14.04**
	12-0168	52	47	73	-111	ND
31.25 mg/kg	12-0170	56	53	105	-146	8
	12-0201[c]					7
	12-0221	51	45	64	-148	38
	12-0240	36	44	62	-71	ND
	12-0299[a]					
	12-0300	32	30	69	-95	27
	12-0346	33	39	67	-98	31
	12-0367	40	36	67	-105	-9
	12-0369	41	38	80	-124	14
	Mean	**37.9**	**36.9**	**65.2**	**-99.8**	**14.5**
	SD	**16.62**	**15.38**	**27.72**	**44.74**	**16.22**
	12-0169	54	45	73	-161	49
125 mg/kg	12-0210	51	39	55	-44	1
	12-0212	47	44	71	-89	2
	12-0227	50	46	67	-100	0
	12-0257	48	39	74	-109	27
	12-0259	52	38	65	-132	-30
	12-0261	50	51	75	-118	33
	12-0278	61	55	105	-114	-9
	12-0291	33	49	66	-127	21
	12-0327	40	37	68	-89	-6
	Mean	**48.6**	**44.3**	**71.9**	**-108.3**	**8.8**
	SD	**7.63**	**6.09**	**12.99**	**31.21**	**23.35**
	12-0166	41	-5	84	-67	5
500 mg/kg	12-0167	40	35	72	-97	23
	12-0171[a]					
	12-0198	51	38	86	-113	25
	12-0229[a]					
	12-0258	57	49	63	-122	35
	12-0279	39	51	34	-108	10
	12-0308[c]					16
	12-0336	44	46	68	-95	ND
	12-0348	41	52	90	-97	-2
	Mean	**44.7**	**38.0**	**71.0**	**-99.9**	**16.0**
	SD	**6.75**	**20.03**	**19.14**	**17.55**	**12.73**

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.
ND = No data

Table F-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Body Mass Gains (grams)
Recovery Male Rats

		Dosing Period				Recovery Period			
Group	Animal ID	Days 0-7	Days 7-14	Days 14-21	Days 21-28	Days 28-35	Days 35-42	Days 42-49	Days 49-55
Corn Oil Control	12-0174	57	60	53	47	33	21	20	15
	12-0179	75	54	58	52	28	13	32	18
	12-0194	54	43	54	59	32	13	30	15
	12-0208	62	53	40	51	21	26	21	17
	12-0272	59	51	43	44	15	17	29	22
	12-0294	54	59	35	42	21	25	28	8
	12-0304	56	51	38	43	16	31	23	17
	12-0323	57	49	44	39	33	30	29	7
	12-0332	41	37	30	35	15	16	20	18
	12-0365	53	50	30	22	26	31	8	13
	Mean	**56.8**	**50.7**	**42.5**	**43.4**	**24.0**	**22.3**	**24.0**	**15.0**
	SD	**8.46**	**6.85**	**9.89**	**10.21**	**7.38**	**7.26**	**7.18**	**4.62**
500 mg/kg	12-0176	55	51	40	45	26	14	19	11
	12-0186	71	48	42	38	12	27	21	5
	12-0215	58	54	52	39	18	11	38	17
	12-0244	49	46	34	30	24	9	16	6
	12-0283	43	38	31	37	21	26	23	14
	12-0295	58	50	41	29	23	36	28	19
	12-0302	47	56	43	39	39	33	30	18
	12-0310	43	50	34	44	38	32	19	27
	12-0333	44	38	42	40	11	32	24	25
	12-0363	59	37	35	36	26	21	13	21
	Mean	**52.7**	**46.8**	**39.4**	**37.7**	**23.8**	**24.1**	**23.1**	**16.3**
	SD	**9.10**	**6.89**	**6.11**	**5.17**	**9.35**	**9.83**	**7.34**	**7.38**

Table F-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Summary of Body Mass Gains (grams)
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Days 0-7	Mean	58.1	55.6	50.7	49.4
	S.D.	4.91	10.15	5.69	12.99
	N	10	10	10	10
Days 7-14	Mean	53.0	47.9	42.0	40.7
	S.D.	5.72	6.95	6.74	14.94
	N	10	10	10	10
Days 14-21	Mean	33.2	27.9	28.8	27.4
	S.D.	6.84	9.66	6.72	14.66
	N	10	9	10	10
Days 21-27	Mean	35.9	32.7	32.2	36.1
	S.D.	4.36	10.26	7.78	8.14
	N	10	9	10	10

Table F-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Summary of Body Mass Gains (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil		
			31.25 mg/kg	125 mg/kg	500 mg/kg
Days 0-7	Mean	13.1	19.0	14.0	10.2[a]
	S.D.	8.02	7.35	7.91	9.99
	N	10	10	10	10
Days 7-14	Mean	17.5	19.2	13.1	12.9[a]
	S.D.	4.88	6.27	6.09	7.81
	N	10	10	10	10

[a] = Significantly reduced compared to 31.25 mg/kg-day group (p=0.006).

Table F-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Gestational and Postpartum Body Mass Gains (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Gestational Days					
Days 0-7	Mean	44.8	37.9	48.6	44.7
	S.D.	6.45	16.62	7.63	6.75
	N	8	8	10	7
Days 7-14	Mean	44.4	36.9	44.3	38.0
	S.D.	5.13	15.38	6.09	20.03
	N	8	8	10	7
Days 14-20	Mean	74.1	65.2	71.9	71.0
	S.D.	5.67	27.72	12.99	19.14
	N	8	8	10	7
Day 20 - PP Day 0	Mean	-111.8	-99.8	-108.3	-99.9
	S.D.	31.24	44.74	31.21	17.55
	N	8	8	10	7
Postpartum Days					
Days 0-4	Mean	8.1	14.5	8.8	16.0
	S.D.	14.04	16.22	23.35	12.73
	N	7	7	10	7

Table F-8
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Body Mass Gains (grams)
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Dosing Period			
Days 0-7	Mean	56.8	52.7
	S.D.	8.46	9.10
	N	10	10
Days 7-14	Mean	50.7	46.8
	S.D.	6.85	6.89
	N	10	10
Days 14-21	Mean	42.5	39.4
	S.D.	9.89	6.11
	N	10	10
Days 21-28	Mean	43.4	37.7
	S.D.	10.21	5.17
	N	10	10
Recovery Period			
Days 28-35	Mean	24.0	23.8
	S.D.	7.38	9.35
	N	10	10
Days 35-42	Mean	22.3	24.1
	S.D.	7.26	9.83
	N	10	10
Days 42-49	Mean	24.0	23.1
	S.D.	7.18	7.34
	N	10	10
Days 49-55	Mean	15.0	16.3
	S.D.	4.62	7.38
	N	10	10

Appendix G

Individual and Summary of Food Consumption

Table G-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Food Consumption (grams)
Male Rats

Group	Animal ID	Days 0-7	Days 7-14	Days 14-21	Days 21-27
Corn Oil Control	12-0163	217.5	209.1	P	153.5
	12-0205	184.8	184.8	P	154.0
	12-0206	190.0	187.9	P	160.4
	12-0207	294.6	382.4*	P	346.5*
	12-0235	245.4	242.1	P	211.2
	12-0254	220.7	212.2	P	174.4
	12-0263	200.1	204.5	P	176.6
	12-0267	217.4	220.7	P	P
	12-0284	220.0	231.3	P	167.7
	12-0313	212.4	225.6	P	160.5
	Mean	**220.29**	**213.13**		**169.79**
	SD	**31.297**	**19.082**		**18.807**
31.25 mg/kg	12-0164	194.3	187.1	P	140.5
	12-0165	201.2	191.0	P	172.1
	12-0225	210.9	207.2	P	176.6
	12-0234	186.0	169.9	P	P
	12-0243	202.6	205.0	P	160.0
	12-0245	198.9	207.4	P	171.9
	12-0255	191.3	195.1	P	P
	12-0262	226.9	226.1	P	186.2
	12-0324	262.4	246.4	P	185.4
	12-0351	224.0	221.9	P	(f)
	Mean	**209.85**	**205.71**		**170.39**
	SD	**22.744**	**21.850**		**15.918**
125 mg/kg	12-0187	212.3	203.1	P	171.0
	12-0204	ND	167.8	P	162.0
	12-0223	226.8	226.7	P	187.9
	12-0224	222.0	208.0	P	184.1
	12-0253	183.8	182.2	P	151.6
	12-0275	204.5	200.4	P	159.0
	12-0293	204.1	202.9	P	145.1
	12-0296	190.0	179.7	P	147.8
	12-0345	211.7	198.4	P	157.3
	12-0354	198.0	194.7	P	167.9
	Mean	**205.91**	**196.39**		**163.37**
	SD	**13.247**	**15.688**		**13.721**
500 mg/kg	12-0177	247.3	250.6	P	P
	12-0189	208.9	213.2	P	173.9
	12-0203	200.2	199.3	P	174.2
	12-0209	214.6	226.9	P	P
	12-0236	236.9	203.7	P	191.4
	12-0256	192.9	150.4	P	160.2
	12-0297	244.8	200.0	P	177.4
	12-0314	199.4	210.4	P	161.6
	12-0322	165.7	179.5	P	112.7
	12-0341	210.8	188.6	P	164.7
	Mean	**212.15**	**202.26**		**164.51**
	SD	**25.311**	**26.956**		**23.246**

* Animal crumbling food on cage floor.
Suspect food consumption
(f) = Animal died on study.
P = Animal pair-housed. No food consumption

Table G-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Pre-Pregnancy Individual Food Consumption (grams)
Female Rats

Group	Animal ID	Days 0-7	Days 7-14	Net
Corn Oil Control	12-0211	134.8	131.9	266.7
	12-0220	130.7	130.8	261.5
	12-0222	118.0	134.6	252.6
	12-0260	114.7	123.7	238.4
	12-0289	133.7	137.7	271.4
	12-0298	132.6	123.3	255.9
	12-0306	122.4	118.9	241.3
	12-0309	113.8	113.0	226.8
	12-0317	145.0	142.7	287.7
	12-0339	125.5	121.6	247.1
	Mean	**127.12**	**127.82**	**254.94**
	SD	**10.025**	**9.224**	**17.779**
31.25 mg/kg	12-0168	149.8	138.4	288.2
	12-0170	141.3	139.3	280.6
	12-0201	132.7	141.4	274.1
	12-0221	127.0	128.9	255.9
	12-0240	123.8	118.3	242.1
	12-0299	140.7	138.8	279.5
	12-0300	128.0	126.0	254
	12-0346	116.5	120.7	237.2
	12-0367	137.6	134.7	272.3
	12-0369	127.0	124.1	251.1
	Mean	**132.44**	**131.06**	**263.50**
	SD	**9.902**	**8.503**	**17.637**
125 mg/kg	12-0169	121.3	111.2	232.5
	12-0210	135.0	122.3	257.3
	12-0212	121.1	118.4	239.5
	12-0227	139.8	139.8	279.6
	12-0257	103.8	111.7	215.5
	12-0259	119.7	122.5	242.2
	12-0261	126.3	121.9	248.2
	12-0278	120.2	136.1	256.3
	12-0291	122.1	120.2	242.3
	12-0327	137.8	138.8	276.6
	Mean	**124.71**	**124.29**	**249.00**
	SD	**10.107**	**9.923**	**19.427**
500 mg/kg	12-0166	118.4	122.2	240.6
	12-0167	119.7	114.1	233.8
	12-0171	130.2	106.3	236.5
	12-0198	134.2	130.0	264.2
	12-0229	109.1	119.5	228.6
	12-0258	113.5	124.6	238.1
	12-0279	109.2	128.1	237.3
	12-0308	131.3	118.5	249.8
	12-0336	110.1	85.3	195.4
	12-0348	123.5	95.9	219.4
	Mean	**119.92**	**114.45**	**234.37**
	SD	**9.568**	**14.505**	**18.166**

Table G-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Gestational and Postpartum Individual Food Consumption (grams)
Female Rats

		Gestational Days				Postpartum Days
Group	Animal ID	Days 0-7	Days 7-14	Days 14-20	Days 20-PP 0	Days 0-4
Corn Oil Control	12-0211	156.4	180.1	167.1	57.4	105.4
	12-0220	153.5	182.9	160.4	8.1	107.2
	12-0222	153	175.8	163.9	32.6	134.8
	12-0260	168.7	199.7	168.8	58.6	ND
	12-0289	178.3	192.4	159	22	124.2
	12-0298[a]					
	12-0306	147.3	162	138.9	11.7	65.4
	12-0309	147.6	176.6	144.7	8.5	94.9
	12-0317[b]					
	12-0339	137.1	154.8	145.3	4.6	30.3
	Mean	**155.24**	**178.04**	**156.01**	**25.44**	**94.60**
	SD	**12.944**	**14.676**	**11.417**	**22.022**	**35.951**
31.25 mg/kg	12-0168	184.6	203.1	173.5	33.8	ND
	12-0170	172.6	197.9	183	24.7	130.4
	12-0201[c]					119.8
	12-0221	165.5	187.7	145.4	11.2	89.9
	12-0240	138.9	155.7	136.4	31.8	ND
	12-0299[a]					
	12-0300	143.7	158.6	153.1	31.6	111.1
	12-0346	140.2	155.8	146.1	23.9	117.6
	12-0367	156	163.3	149.5	6.7	75.5
	12-0369	162.8	165.1	139	8.7	105.3
	Mean	**158.04**	**173.40**	**153.25**	**21.55**	**107.09**
	SD	**16.432**	**19.639**	**16.514**	**11.112**	**18.833**
125 mg/kg	12-0169	160.8	174	152.6	10.6	55.2
	12-0210	170.1	200	185.2	ND	106.3
	12-0212	157.5	178.8	165	61	78.3
	12-0227	194	214.2	157.7	26.4	110
	12-0257	149.2	162.8	166.7	16.5	134.2
	12-0259	171.9	181.5	150	5.2	5.1
	12-0261	169.9	188.1	171	40.1	150.7
	12-0278	189.6	215	205	19.5	103
	12-0291	138.1	163.2	162.3	82	148.5
	12-0327	155.5	174.2	153.9	30	77.8
	Mean	**165.66**	**185.18**	**166.94**	**32.37**	**96.91**
	SD	**17.246**	**18.983**	**16.904**	**25.034**	**44.873**
500 mg/kg	12-0166	149.7	122.3	136.7	51.3	101.9
	12-0167	141.2	148.6	143	52.2	83.8
	12-0171[a]					
	12-0198	167.2	187.8	160	30.1	143.5
	12-0229[a]					
	12-0258	179.7	199.1	159	11.1	62.6
	12-0279	156.6	191.3	96.2	23.1	87.4
	12-0308[c]					134.6
	12-0336	132.2	158.8	140.8	20.3	ND
	12-0348	148.4	173	165	31.3	106.8
	Mean	**153.57**	**168.70**	**142.96**	**31.34**	**102.94**
	SD	**15.964**	**27.304**	**23.323**	**15.462**	**28.573**

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but dam was pregnant.
ND= No data

Table G-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Food Consumption (grams)
Recovery Male Rats

		Dosing Period				Recovery Period				
Group	Animal ID	Days 0-7	Days 7-14	Days 14-21	Days 21-28	Days 28-35	Days 35-42	Days 42-49	Days 49-55	Net
Corn Oil Control	12-0174	196.9	208.2	213.1	217.8	242.1	240.2	234.5	202.2	1755.0
	12-0179	238.3	236.0	247.8	243.2	271.4	237.7	245.5	202.0	1921.9
	12-0194	213.1	208.0	222.3	222.3	256.4	242.6	238.8	199.7	1803.2
	12-0208	205.2	201.7	189.5	200.3	214.0	218.5	220.4	184.1	1633.7
	12-0272	229.8	222.3	221.7	217.4	225.1	224.3	247.1	199.9	1787.6
	12-0294	218.6	222.5	206.5	212.3	224.7	214.9	222.4	180.6	1702.5
	12-0304	212.7	195.1	196.9	197.8	207.4	218.3	218.8	187.2	1634.2
	12-0323	214.2	207.8	210.2	203.8	237.9	246.8	246.4	192.1	1759.2
	12-0332	186.2	183.6	184.0	193.3	206.6	210.1	217.8	179.4	1561.0
	12-0365	229.1	217.0	205.8	193.7	215.1	225.2	220.7	184.8	1691.4
	Mean	**214.41**	**210.22**	**209.78**	**210.19**	**230.07**	**227.86**	**231.24**	**191.20**	**1724.97**
	SD	**15.751**	**15.026**	**18.330**	**15.635**	**21.623**	**12.941**	**12.448**	**9.100**	**103.271**
500 mg/kg	12-0176	232.9	222.3	219.7	214.8	231.1	225.3	228.1	183.1	1757.3
	12-0186	225.3	211.2	200.0	191.5	211.6	212.1	209.2	171.2	1632.1
	12-0215	216.8	219.4	215.4	216.5	226.4	218.2	239.1	191.5	1743.3
	12-0244	199.6	198.1	199.5	192.6	206.6	197.2	199.3	159.3	1552.2
	12-0283	180.5	173.7	172.8	164.5	191.7	194.7	202.3	168.2	1448.4
	12-0295	205.0	201.6	206.3	178.1	202.8	222.5	225.2	193.3	1634.8
	12-0302	223.2	215.8	214.8	208.6	241.4	253.4	264.4	208.4	1830.0
	12-0310	208.4	201.7	185.4	196.6	226.0	226.9	243.0	202.9	1690.9
	12-0333	191.7	183.3	194.7	196.0	215.9	216.4	225.3	195.6	1618.9
	12-0363	228.4	209.5	211.9	202.9	224.3	223.0	218.3	192.2	1710.5
	Mean	**211.18**	**203.66**	**202.05**	**196.21**	**217.78**	**218.97**	**225.42**	**186.57**	**1661.84**
	SD	**17.141**	**15.580**	**14.776**	**16.071**	**14.874**	**16.447**	**19.870**	**15.830**	**109.684**

Table G-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Food Consumption (grams)
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Days 0-7	Mean	220.29	209.85	205.91	212.15
	S.D.	31.297	22.744	13.247	25.311
	N	10	10	9	10
Days 7-14	Mean	213.13	205.71	196.39	202.26
	S.D.	19.082	21.850	15.688	26.956
	N	9	10	10	10
Days 14-21	Mean	*	*	*	*
	S.D.				
	N	0	0	0	0
Days 21-27	Mean	169.79	170.39	163.37	164.51
	S.D.	18.807	15.918	13.721	23.246
	N	8	7	10	8

* = All animals were pair-housed for mating during this period.

Table G-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Food Consumption (grams)
Female Rats - Pre-Pregnancy

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Days 0-7	Mean	127.12	132.44	124.71	119.92
	S.D.	10.025	9.902	10.107	9.568
	N	10	10	10	10
Days 7-14	Mean	127.82	131.06	124.29	114.45
	S.D.	9.224	8.503	9.923	14.505
	N	10	10	10	10
Net	Mean	254.94	263.50	249.00	234.37[a]
	S.D.	17.779	17.637	19.427	18.166
	N	10	10	10	10

[a] = Significantly reduced compared to 31.25 mg/kg-day group (p=0.006).

Table G-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Gestational and Postpartum Food Consumption (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Gestational Days					
Days 0-7	Mean	155.24	158.04	165.66	153.57
	S.D.	12.944	16.432	17.246	15.964
	N	8	8	10	7
Days 7-14	Mean	178.04	173.40	185.18	168.70
	S.D.	14.676	19.639	18.983	27.304
	N	8	8	10	7
Days 14-20	Mean	156.01	153.25	166.94	142.96
	S.D.	11.417	16.514	16.904	23.323
	N	8	8	10	7
Days 20- PP 0	Mean	25.44	21.55	32.37	31.34
	S.D.	22.022	11.112	25.034	15.462
	N	8	8	10	7
Postpartum Days					
Days 0-4	Mean	94.60	107.09	96.91	102.94
	S.D.	35.951	18.833	44.873	28.573
	N	7	7	10	7

Table G-8
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Food Consumption (grams)
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Days 0-7	Mean	214.41	211.18
	S.D.	15.751	17.141
	N	10	10
Days 7-14	Mean	201.22	203.66
	S.D.	15.026	15.580
	N	10	10
Days 14-21	Mean	209.78	202.05
	S.D.	18.330	14.776
	N	10	10
Days 21-28	Mean	210.19	196.21
	S.D.	15.635	16.071
	N	10	10
Days 28-35	Mean	230.07	217.78
	S.D.	21.623	14.874
	N	10	10
Days 35-42	Mean	227.86	218.97
	S.D.	12.941	16.447
	N	10	10
Days 42-49	Mean	231.24	225.42
	S.D.	12.448	19.870
	N	10	10
Days 49-55	Mean	191.20	186.57
	S.D.	9.100	15.830
	N	10	10
Net	Mean	1724.97	1661.84
	S.D.	103.271	109.684
	N	10	10

Appendix H

Individual and Summary of Organ Mass Data

Table H-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Male Rats

ABSOLUTE ORGAN MASS (grams)

Group	Animal ID	Body Weight*	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	12-0163	486	0.068	2.157	1.809	3.532	1.143	19.240	0.866	3.682	0.463
Corn Oil	12-0205	464	0.098	2.112	1.768	3.166	1.311	16.425	0.770	3.489	0.707
Control	12-0206	473	0.091	2.188	1.775	3.240	1.267	17.650	0.795	3.449	0.619
	12-0207	483	0.088	2.036	1.851	3.271	1.256	17.244	0.934	3.933	0.701
	12-0235	572	0.094	2.162	1.902	3.781	1.420	20.204	0.852	3.656	0.759
	12-0254	483	0.077	2.145	1.719	3.161	0.911	15.267	1.011	2.541	0.654
	12-0263	507	0.079	2.143	2.018	3.332	1.263	17.842	0.958	3.794	0.682
	12-0267	540	0.068	2.001	2.274	3.372	1.436	18.032	0.941	3.631	0.638
	12-0284	515	0.118	1.954	1.928	3.712	1.162	18.451	1.378	3.841	0.600
	12-0313	500	0.079	2.364	1.886	3.172	1.600	16.787	0.839	3.519	0.634
	Mean	**502.3**	**0.0860**	**2.1262**	**1.8930**	**3.3739**	**1.2769**	**17.7142**	**0.9344**	**3.5535**	**0.6457**
	SD	**33.01**	**0.01523**	**0.11397**	**0.16013**	**0.22721**	**0.18770**	**1.41119**	**0.17329**	**0.38858**	**0.07987**
	12-0164	452	0.074	2.096	1.656	3.699	1.167	18.725	0.846	3.911	0.522
31.25 mg/kg	12-0165	488	0.077	2.107	1.753	3.420	1.274	19.904	0.770	3.808	0.604
	12-0225	493	0.071	2.297	1.685	3.427	1.253	18.955	0.879	3.867	0.643
	12-0234	440	0.060	2.043	1.416	2.694	1.178	14.380	0.685	3.387	0.660
	12-0243	441	0.081	2.163	1.622	3.064	1.198	15.059	0.791	3.615	0.592
	12-0245	477	0.094	2.111	1.690	3.518	1.207	17.450	0.814	3.502	0.655
	12-0255	425	0.106	2.209	1.731	2.932	1.176	12.456	0.929	3.362	0.522
	12-0262	557	0.079	2.151	2.126	3.906	1.436	19.949	0.966	3.785	0.845
	12-0324	526	0.109	2.218	1.821	3.582	1.147	18.247	0.999	3.432	0.794
	12-0351	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)
	Mean	**477.7**	**0.0834**	**2.155**	**1.7222**	**3.3602**	**1.2262**	**17.2361**	**0.8532**	**3.6299**	**0.6486**
	SD	**43.54**	**0.01633**	**0.07697**	**0.18856**	**0.38887**	**0.08860**	**2.65667**	**0.10066**	**0.21723**	**0.11021**
	12-0187	517	0.079	2.115	1.937	3.285	1.213	17.612	0.903	3.734	0.677
125 mg/kg	12-0204	478	0.082	2.134	1.855	2.949	1.426	17.883	0.995	3.932	0.722
	12-0223	500	0.071	2.213	1.974	3.440	1.300	18.239	0.833	3.667	0.582
	12-0224	488	0.079	2.241	1.580	3.501	1.263	16.797	0.862	3.707	0.654
	12-0253	426	0.091	2.172	1.708	2.936	1.204	14.057	0.879	3.913	0.605
	12-0275	472	0.085	2.268	1.738	3.226	1.386	16.890	0.777	3.840	0.537
	12-0293	473	0.073	2.191	1.748	3.006	1.426	16.286	0.902	3.519	0.506
	12-0296	447	0.082	2.189	1.663	3.043	1.280	14.915	0.792	4.128	0.509
	12-0345	444	0.095	2.221	1.707	3.065	1.394	16.014	0.838	3.798	0.542
	12-0354	494	0.111	2.279	1.829	3.169	1.274	15.964	0.853	3.385	0.609
	Mean	**473.9**	**0.0848**	**2.2023**	**1.7739**	**3.1620**	**1.3166**	**16.4657**	**0.8634**	**3.7623**	**0.5943**
	SD	**28.04**	**0.01175**	**0.05341**	**0.12335**	**0.19884**	**0.08463**	**1.31029**	**0.06224**	**0.21267**	**0.07325**
	12-0177	553	0.091	2.217	1.813	3.263	0.769	18.511	1.169	1.718	0.977
500 mg/kg	12-0189	505	0.063	2.050	1.567	3.629	0.591	17.764	0.895	1.546	0.613
	12-0203	465	0.082	2.147	1.647	3.023	0.702	14.872	0.875	1.442	0.631
	12-0209	513	0.080	2.162	1.961	3.706	0.812	18.999	0.882	1.699	0.543
	12-0236	508	0.104	2.038	1.862	2.920	0.848	18.484	0.882	1.146	0.841
	12-0256	411	0.082	2.114	1.545	2.850	0.812	13.845	0.757	1.425	0.458
	12-0297	515	0.117	2.120	1.781	4.064	1.063	18.519	1.258	1.621	0.631
	12-0314	486	0.120	2.135	1.729	3.472	1.061	ND	0.939	1.393	0.510
	12-0322	382	0.089	2.082	1.585	2.598	0.818	12.167	0.708	1.445	0.571
	12-0341	448	0.084	2.107	1.792	3.255	0.817	16.203	0.883	1.315	0.539
	Mean	**478.6**	**0.0912**	**2.1172**	**1.7282**	**3.2780**	**0.8293**	**16.5960**	**0.9248**	**1.4750**	**0.6314**
	SD	**52.37**	**0.01766**	**0.05304**	**0.13856**	**0.44738**	**0.14382**	**2.45914**	**0.16826**	**0.17658**	**0.15934**

(f) = Animal died on study
* = Final fasted body weight
ND = No data

Table H-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Female Rats

ABSOLUTE ORGAN MASS (grams)

Group	Animal ID	Body Weight*	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus	Implantation Sites	Corpora Lutea
	12-0211	341	0.084	2.020	1.396	2.080	12.634	0.123	0.788	0.392	0.607	8	20
Corn Oil	12-0220	331	0.071	2.096	1.338	1.933	15.635	0.118	0.985	0.455	0.602	17	17
Control	12-0222	323	0.074	2.033	1.244	2.102	12.394	0.137	0.734	0.482	0.664	16	19
	12-0260	266	0.111	1.997	0.947	2.095	9.820	0.173	0.489	0.191	0.794	15	15
	12-0289	287	0.098	1.906	1.207	1.866	11.627	0.146	0.788	0.243	0.582	17	21
	12-0298[a,d]												
	12-0306	270	0.084	2.044	1.114	2.146	11.084	0.142	0.657	0.273	0.848	16	16
	12-0309	300	0.102	2.051	1.207	2.289	13.008	0.182	0.731	0.208	0.669	16	16
	12-0317[b]												
	12-0339	249	0.075	1.890	0.963	1.964	ND	0.214	ND	0.296	0.589	17	18
	Mean	**295.9**	**0.0874**	**2.004625**	**1.1770**	**2.059375**	**12.3146**	**0.154375**	**0.7389**	**0.3175**	**0.669375**	**15.3**	**17.8**
	SD	**33.51**	**0.01468**	**0.07173**	**0.16162**	**0.13427**	**1.81771**	**0.03273**	**0.14979**	**0.11180**	**0.09999**	**3.01**	**2.12**
	12-0168	336	0.104	1.972	1.359	2.486	11.802	0.152	0.649	0.348	0.850	16	16
31.25 mg/kg	12-0170	329	0.104	1.921	1.393	2.241	15.412	0.181	0.812	0.236	0.662	20	20
	12-0201[c]	317	0.067	1.966	1.107	2.103	13.134	0.127	0.593	0.185	0.486	17	18
	12-0221	325	0.078	2.013	1.158	1.969	14.391	0.138	0.648	0.096	0.722	18	19
	12-0240	299	0.053	1.862	1.038	1.692	10.906	0.156	0.544	0.288	0.618	12	12
	12-0299[a]												
	12-0300	293	0.096	1.935	1.150	2.103	12.102	0.165	0.598	0.282	0.838	13	15
	12-0346	261	0.095	1.872	1.045	1.762	10.247	0.154	0.601	0.367	0.598	13	13
	12-0367	278	0.064	1.857	1.001	1.789	9.622	0.126	0.568	0.360	0.551	15	15
	12-0369	293	0.069	1.884	1.162	2.089	11.294	0.100	0.547	0.165	0.602	16	17
	Mean	**303.4**	**0.0811**	**1.920222**	**1.1570**	**2.0260**	**12.1011**	**0.1443**	**0.6178**	**0.2586**	**0.6586**	**15.6**	**16.1**
	SD	**25.12**	**0.01904**	**0.05548**	**0.13698**	**0.25356**	**1.90478**	**0.02424**	**0.08203**	**0.09524**	**0.12387**	**2.60**	**2.67**
	12-0169	289	0.163	1.931	1.120	2.304	12.178	0.140	0.742	0.118	0.968	17	23
125 mg/kg	12-0210	344	0.064	1.989	1.479	2.186	11.950	0.125	0.774	0.435	0.656	16	16
	12-0212	303	0.091	1.976	1.123	1.932	11.641	0.173	0.614	0.197	0.616	13	13
	12-0227	318	0.088	2.123	1.315	1.995	11.766	0.152	0.547	0.310	0.718	15	15
	12-0257	293	0.090	2.006	1.170	1.677	11.089	0.163	0.671	0.141	0.720	15	15
	12-0259	238	0.106	1.993	1.015	2.102	8.831	0.181	0.484	0.217	0.781	18	19
	12-0261	313	0.091	2.001	1.226	2.110	13.261	0.185	0.660	0.338	0.696	17	17
	12-0278	343	0.102	2.089	1.560	2.511	13.849	0.134	0.591	0.320	0.757	18	18
	12-0291	283	0.084	1.812	1.042	2.294	11.547	0.137	0.573	0.179	0.719	14	14
	12-0327	293	0.101	2.006	1.021	2.284	10.944	0.138	0.638	0.291	0.891	12	16
	Mean	**301.7**	**0.0980**	**1.9926**	**1.2071**	**2.1395**	**11.7056**	**0.1528**	**0.6294**	**0.2546**	**0.7522**	**15.5**	**16.6**
	SD	**30.93**	**0.02566**	**0.08395**	**0.19012**	**0.23383**	**1.35617**	**0.02135**	**0.08766**	**0.10010**	**0.10591**	**2.07**	**2.88**
	12-0166	294	0.073	1.905	1.108	1.706	11.092	0.124	0.623	0.228	0.623	16	25
500 mg/kg	12-0167	322	0.069	1.995	1.061	2.139	12.561	0.111	0.616	0.234	0.699	19	33
	12-0171[a]												
	12-0198	319	0.090	2.006	1.205	2.062	12.532	0.142	0.670	0.159	0.487	14	16
	12-0229[a]												
	12-0258	288	0.096	1.936	1.154	1.920	10.720	0.196	0.535	0.375	1.385[*3]	17	17
	12-0279	275	0.079	2.051	1.057	2.239	9.864	0.123	0.398	0.218	0.503	16	16
	12-0308[c]	323	0.093	2.083	1.525	2.265	11.814	0.197	0.716	0.323	0.681	12	23
	12-0336	262	0.033[*2]	1.834	1.006	1.779	9.729	0.122	0.490	0.253	0.478	13	13
	12-0348	323	0.058	2.072	1.169	2.219	11.209	0.122	0.611	0.295	0.633	15	15
	Mean	**300.8**	**0.0797**	**1.9853**	**1.1606**	**2.0411**	**11.1901**	**0.1421**	**0.5824**	**0.2606**	**0.5863**	**15.3**	**19.8**
	SD	**24.34**	**0.01402**	**0.08756**	**0.16133**	**0.21601**	**1.08191**	**0.03461**	**0.10275**	**0.06775**	**0.09463**	**2.25**	**6.73**

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.
d= Animal was not fasted prior to necropsy.
* = Fasted final body weights.
*2= Only 1 adrenal weight was obtained.
*3 = Identified as outlier and dropped from analysis.
ND = No data

Table H-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Recovery Male Rats

ABSOLUTE ORGAN MASS (grams)

Group	Animal ID	Body Weight*	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
Corn Oil Control	12-0174	618	0.059	2.219	2.055	3.624	1.427	22.590	1.245	4.031	0.459
	12-0179	653	0.062	2.378	2.282	4.042	1.350	24.950	1.567	3.871	0.554
	12-0194	663	0.073	2.294	2.234	4.319	1.354	24.909	1.044	3.457	0.489
	12-0208	595	0.082	2.286	1.784	3.732	1.442	19.590	0.929	3.411	0.670
	12-0272	617	0.081	2.352	2.041	4.023	1.429	23.169	1.027	3.535	0.529
	12-0294	588	0.064	2.166	2.061	3.644	1.189	19.069	1.148	3.679	0.455
	12-0304	625	0.068	2.226	1.979	3.959	1.106	19.694	1.103	3.896	0.493
	12-0323	572	0.070	2.114	1.818	3.558	0.920	21.455	0.956	3.157	0.396
	12-0332	482	0.068	2.228	1.986	3.634	1.157	17.010	0.814	3.385	0.336
	12-0365	558	0.050	2.175	1.795	3.596	1.194	18.963	0.984	3.520	0.415
	Mean	**597.1**	**0.0677**	**2.2438**	**2.0035**	**3.8131**	**1.2568**	**21.1399**	**1.0817**	**3.5942**	**0.4796**
	SD	**52.25**	**0.00972**	**0.08356**	**0.17160**	**0.25580**	**0.17183**	**2.70018**	**0.20864**	**0.27121**	**0.09268**
500 mg/kg	12-0176	611	0.061	2.242	1.929	3.708	0.771	22.502	1.338	1.707	0.406
	12-0186	599	0.058	2.243	2.079	3.985	0.959	21.862	1.163	2.543	0.496
	12-0215	614	0.056	2.394	2.100	3.601	0.872	20.602	1.549	2.212	0.636
	12-0244	510	0.072	2.103	1.663	3.412	0.807	16.128	0.865	2.577	0.428
	12-0283	510	0.071	2.203	1.916	3.880	0.439	17.906	1.066	1.885	0.381
	12-0295	602	0.051*[1]	2.188	1.960	3.929	0.477	19.711	1.276	2.517	0.468
	12-0302	664	0.072	2.446	2.049	4.106	0.542	22.938	1.097	2.317	0.381
	12-0310	621	0.083	2.336	1.938	3.824	0.683	21.591	1.062	2.126	0.469
	12-0333	528	0.077	2.090	1.827	3.702	0.739	17.057	0.863	1.862	0.459
	12-0363	561	0.064	2.158	2.039	3.452	0.738	17.299	1.082	1.775	0.545
	Mean	**582.0**	**0.0682**	**2.2403**	**1.9500**	**3.7599**	**0.7027**	**19.7596**	**1.1361**	**2.1521**	**0.4669**
	SD	**52.20**	**0.00905**	**0.11900**	**0.13174**	**0.22723**	**0.16969**	**2.49649**	**0.20936**	**0.33229**	**0.07859**

* = Final fasted body weight
*1 = Only 1 adrenal weighed

Table H-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Male Rats

ORGAN MASS RELATIVE TO BODY MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	12-0163	0.00014	0.0044	0.0037	0.0073	0.0024	0.0396	0.0018	0.0076	0.0010
Corn Oil	12-0205	0.00021	0.0046	0.0038	0.0068	0.0028	0.0354	0.0017	0.0075	0.0015
Control	12-0206	0.00019	0.0046	0.0038	0.0068	0.0027	0.0373	0.0017	0.0073	0.0013
	12-0207	0.00018	0.0042	0.0038	0.0068	0.0026	0.0357	0.0019	0.0081	0.0015
	12-0235	0.00016	0.0038	0.0033	0.0066	0.0025	0.0353	0.0015	0.0064	0.0013
	12-0254	0.00016	0.0044	0.0036	0.0065	0.0019	0.0316	0.0021	0.0053	0.0014
	12-0263	0.00016	0.0042	0.0040	0.0066	0.0025	0.0352	0.0019	0.0075	0.0013
	12-0267	0.00013	0.0037	0.0042	0.0062	0.0027	0.0334	0.0017	0.0067	0.0012
	12-0284	0.00023	0.0038	0.0037	0.0072	0.0023	0.0358	0.0027	0.0075	0.0012
	12-0313	0.00016	0.0047	0.0038	0.0063	0.0032	0.0336	0.0017	0.0070	0.0013
	Mean	**0.000172**	**0.00425**	**0.00377**	**0.00672**	**0.00254**	**0.03529**	**0.00186**	**0.00709**	**0.00129**
	SD	**3.188E-05**	**0.000374**	**0.000233**	**0.000333**	**0.000350**	**0.002190**	**0.000331**	**0.000805**	**0.000160**
	12-0164	0.00016	0.0046	0.0037	0.0082	0.0026	0.0414	0.0019	0.0087	0.0012
31.25 mg/kg	12-0165	0.00016	0.0043	0.0036	0.0070	0.0026	0.0408	0.0016	0.0078	0.0012
	12-0225	0.00014	0.0047	0.0034	0.0070	0.0025	0.0384	0.0018	0.0078	0.0013
	12-0234	0.00014	0.0046	0.0032	0.0061	0.0027	0.0327	0.0016	0.0077	0.0015
	12-0243	0.00018	0.0049	0.0037	0.0069	0.0027	0.0341	0.0018	0.0082	0.0013
	12-0245	0.00020	0.0044	0.0035	0.0074	0.0025	0.0366	0.0017	0.0073	0.0014
	12-0255	0.00025	0.0052	0.0041	0.0069	0.0028	0.0293	0.0022	0.0079	0.0012
	12-0262	0.00014	0.0039	0.0038	0.0070	0.0026	0.0358	0.0017	0.0068	0.0015
	12-0324	0.00021	0.0042	0.0035	0.0068	0.0022	0.0347	0.0019	0.0065	0.0015
	12-0351	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)
	Mean	**0.000176**	**0.00454**	**0.00361**	**0.00703**	**0.00258**	**0.03599**	**0.00179**	**0.00764**	**0.00135**
	SD	**3.727E-05**	**0.000392**	**0.000245**	**0.000542**	**0.000169**	**0.003868**	**0.000189**	**0.000664**	**0.000134**
	12-0187	0.00015	0.0041	0.0037	0.0064	0.0023	0.0341	0.0017	0.0072	0.0013
125 mg/kg	12-0204	0.00017	0.0045	0.0039	0.0062	0.0030	0.0374	0.0021	0.0082	0.0015
	12-0223	0.00014	0.0044	0.0039	0.0069	0.0026	0.0365	0.0017	0.0073	0.0012
	12-0224	0.00016	0.0046	0.0032	0.0072	0.0026	0.0344	0.0018	0.0076	0.0013
	12-0253	0.00021	0.0051	0.0040	0.0069	0.0028	0.0330	0.0021	0.0092	0.0014
	12-0275	0.00018	0.0048	0.0037	0.0068	0.0029	0.0358	0.0016	0.0081	0.0011
	12-0293	0.00015	0.0046	0.0037	0.0064	0.0030	0.0344	0.0019	0.0074	0.0011
	12-0296	0.00018	0.0049	0.0037	0.0068	0.0029	0.0334	0.0018	0.0092	0.0011
	12-0345	0.00021	0.0050	0.0038	0.0069	0.0031	0.0361	0.0019	0.0086	0.0012
	12-0354	0.00022	0.0046	0.0037	0.0064	0.0026	0.0323	0.0017	0.0069	0.0012
	Mean	**0.000180**	**0.00466**	**0.00375**	**0.00668**	**0.00279**	**0.03473**	**0.00183**	**0.00798**	**0.00125**
	SD	**2.893E-05**	**0.0003**	**0.000212**	**0.000327**	**0.000249**	**0.001649**	**0.000154**	**0.000826**	**0.000139**
	12-0177	0.00016	0.0040	0.0033	0.0059	0.0014	0.0335	0.0021	0.0031	0.0018
500 mg/kg	12-0189	0.00012	0.0041	0.0031	0.0072	0.0012	0.0352	0.0018	0.0031	0.0012
	12-0203	0.00018	0.0046	0.0035	0.0065	0.0015	0.0320	0.0019	0.0031	0.0014
	12-0209	0.00016	0.0042	0.0038	0.0072	0.0016	0.0370	0.0017	0.0033	0.0011
	12-0236	0.00020	0.0040	0.0037	0.0057	0.0017	0.0364	0.0017	0.0023	0.0017
	12-0256	0.00020	0.0051	0.0038	0.0069	0.0020	0.0337	0.0018	0.0035	0.0011
	12-0297	0.00023	0.0041	0.0035	0.0079	0.0021	0.0360	0.0024	0.0031	0.0012
	12-0314	0.00025	0.0044	0.0036	0.0071	0.0022	ND	0.0019	0.0029	0.0010
	12-0322	0.00023	0.0055	0.0041	0.0068	0.0021	0.0319	0.0019	0.0038	0.0015
	12-0341	0.00019	0.0047	0.0040	0.0073	0.0018	0.0362	0.0020	0.0029	0.0012
	Mean	**0.000192**	**0.00447**	**0.00363**	**0.00686**	**0.00175**	**0.03464**	**0.00193**	**0.00310**	**0.00131**
	SD	**3.799E-05**	**0.000503**	**0.000317**	**0.000653**	**0.000342**	**0.001945**	**0.000216**	**0.000401**	**0.000250**

(f) = Animal died on study
ND = No data

Table H-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Female Rats

ORGAN MASS RELATIVE TO BODY MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
Corn Oil Control	12-0211	0.0002	0.0059	0.0041	0.0061	0.0370	0.0004	0.0023	0.0011	0.0018
	12-0220	0.0002	0.0063	0.0040	0.0058	0.0472	0.0004	0.0030	0.0014	0.0018
	12-0222	0.0002	0.0063	0.0039	0.0065	0.0384	0.0004	0.0023	0.0015	0.0021
	12-0260	0.0004	0.0075	0.0036	0.0079	0.0369	0.0007	0.0018	0.0007	0.0030
	12-0289	0.0003	0.0066	0.0042	0.0065	0.0405	0.0005	0.0027	0.0008	0.0020
	12-0298[a]									
	12-0306	0.0003	0.0076	0.0041	0.0079	0.0411	0.0005	0.0024	0.0010	0.0031
	12-0309	0.0003	0.0068	0.0040	0.0076	0.0434	0.0006	0.0024	0.0007	0.0022
	12-0317[b]									
	12-0339	0.0003	0.0076	0.0039	0.0079	ND	0.0009	ND	0.0012	0.0024
	Mean	**0.00030**	**0.00684**	**0.00397**	**0.00704**	**0.04064**	**0.00054**	**0.00243**	**0.00106**	**0.00230**
	SD	**0.000068**	**0.000652**	**0.000206**	**0.000885**	**0.003719**	**0.000169**	**0.000362**	**0.000295**	**0.000510**
31.25 mg/kg	12-0168	0.0003	0.0059	0.0040	0.0074	0.0351	0.0005	0.0019	0.0010	0.0025
	12-0170	0.0003	0.0058	0.0042	0.0068	0.0468	0.0006	0.0025	0.0007	0.0020
	12-0201[c]	0.0002	0.0062	0.0035	0.0066	0.0414	0.0004	0.0019	0.0006	0.0015
	12-0221	0.0002	0.0062	0.0036	0.0061	0.0443	0.0004	0.0020	0.0003	0.0022
	12-0240	0.0002	0.0062	0.0035	0.0057	0.0365	0.0005	0.0018	0.0010	0.0021
	12-0299[a]									
	12-0300	0.0003	0.0066	0.0039	0.0072	0.0413	0.0006	0.0020	0.0010	0.0029
	12-0346	0.0004	0.0072	0.0040	0.0068	0.0393	0.0006	0.0023	0.0014	0.0023
	12-0367	0.0002	0.0067	0.0036	0.0064	0.0346	0.0005	0.0020	0.0013	0.0020
	12-0369	0.0002	0.0064	0.0040	0.0071	0.0385	0.0003	0.0019	0.0006	0.0021
	Mean	**0.00027**	**0.00636**	**0.00381**	**0.00667**	**0.03976**	**0.00048**	**0.00204**	**0.00087**	**0.00217**
	SD	**0.000063**	**0.000420**	**0.000281**	**0.000557**	**0.004128**	**0.000083**	**0.000216**	**0.000361**	**0.000373**
125 mg/kg	12-0169	0.0006	0.0067	0.0039	0.0080	0.0421	0.0005	0.0026	0.0004	0.0033
	12-0210	0.0002	0.0058	0.0043	0.0064	0.0347	0.0004	0.0023	0.0013	0.0019
	12-0212	0.0003	0.0065	0.0037	0.0064	0.0384	0.0006	0.0020	0.0007	0.0020
	12-0227	0.0003	0.0067	0.0041	0.0063	0.0370	0.0005	0.0017	0.0010	0.0023
	12-0257	0.0003	0.0068	0.0040	0.0057	0.0378	0.0006	0.0023	0.0005	0.0025
	12-0259	0.0004	0.0084	0.0043	0.0088	0.0371	0.0008	0.0020	0.0009	0.0033
	12-0261	0.0003	0.0064	0.0039	0.0067	0.0424	0.0006	0.0021	0.0011	0.0022
	12-0278	0.0003	0.0061	0.0045	0.0073	0.0404	0.0004	0.0017	0.0009	0.0022
	12-0291	0.0003	0.0064	0.0037	0.0081	0.0408	0.0005	0.0020	0.0006	0.0025
	12-0327	0.0003	0.0068	0.0035	0.0078	0.0374	0.0005	0.0022	0.0010	0.0030
	Mean	**0.00033**	**0.00666**	**0.00399**	**0.00715**	**0.03881**	**0.00052**	**0.00209**	**0.00083**	**0.00253**
	SD	**0.000104**	**0.000688**	**0.000325**	**0.001004**	**0.002497**	**0.000113**	**0.000255**	**0.000276**	**0.000517**
500 mg/kg	12-0166	0.0002	0.0065	0.0038	0.0058	0.0377	0.0004	0.0021	0.0008	0.0021
	12-0167	0.0002	0.0062	0.0033	0.0066	0.0390	0.0003	0.0019	0.0007	0.0022
	12-0171[a]									
	12-0198	0.0003	0.0063	0.0038	0.0065	0.0393	0.0004	0.0021	0.0005	0.0015
	12-0229[a]									
	12-0258	0.0003	0.0067	0.0040	0.0067	0.0372	0.0007	0.0019	0.0013	0.0048*
	12-0279	0.0003	0.0075	0.0038	0.0081	0.0359	0.0004	0.0014	0.0008	0.0018
	12-0308[c]	0.0003	0.0064	0.0047	0.0070	0.0366	0.0006	0.0022	0.0010	0.0021
	12-0336	ND	0.0070	0.0038	0.0068	0.0371	0.0005	0.0019	0.0010	0.0018
	12-0348	0.0002	0.0064	0.0036	0.0069	0.0347	0.0004	0.0019	0.0009	0.0020
	Mean	**0.00026**	**0.00663**	**0.00386**	**0.00680**	**0.03719**	**0.00047**	**0.00193**	**0.00087**	**0.00193**
	SD	**0.000052**	**0.000421**	**0.000406**	**0.000655**	**0.001526**	**0.000114**	**0.000236**	**0.000235**	**0.000227**

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but dam was pregnant.
d= Animal was not fasted prior to necropsy.
* = Identified as an outlier.
ND = No data

Table H-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Recovery Male Rats

ORGAN MASS RELATIVE TO BODY MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
	12-0174	0.00010	0.0036	0.0033	0.0059	0.0023	0.0366	0.0020	0.0065	0.0007
Corn Oil	12-0179	0.00009	0.0036	0.0035	0.0062	0.0021	0.0382	0.0024	0.0059	0.0008
Control	12-0194	0.00011	0.0035	0.0034	0.0065	0.0020	0.0376	0.0016	0.0052	0.0007
	12-0208	0.00014	0.0038	0.0030	0.0063	0.0024	0.0329	0.0016	0.0057	0.0011
	12-0272	0.00013	0.0038	0.0033	0.0065	0.0023	0.0376	0.0017	0.0057	0.0009
	12-0294	0.00011	0.0037	0.0035	0.0062	0.0020	0.0324	0.0020	0.0063	0.0008
	12-0304	0.00011	0.0036	0.0032	0.0063	0.0018	0.0315	0.0018	0.0062	0.0008
	12-0323	0.00012	0.0037	0.0032	0.0062	0.0016	0.0375	0.0017	0.0055	0.0007
	12-0332	0.00014	0.0046	0.0041	0.0075	0.0024	0.0353	0.0017	0.0070	0.0007
	12-0365	0.00009	0.0039	0.0032	0.0064	0.0021	0.0340	0.0018	0.0063	0.0007
	Mean	**0.000114**	**0.00378**	**0.00337**	**0.00641**	**0.00211**	**0.03535**	**0.00181**	**0.00605**	**0.00080**
	SD	**1.838E-05**	**0.000325**	**0.000306**	**0.000441**	**0.000268**	**0.002474**	**0.000255**	**0.000528**	**0.000127**
	12-0176	0.00010	0.0037	0.0032	0.0061	0.0013	0.0368	0.0022	0.0028	0.0007
500 mg/kg	12-0186	0.00010	0.0037	0.0035	0.0067	0.0016	0.0365	0.0019	0.0042	0.0008
	12-0215	0.00009	0.0039	0.0034	0.0059	0.0014	0.0336	0.0025	0.0036	0.0010
	12-0244	0.00014	0.0041	0.0033	0.0067	0.0016	0.0316	0.0017	0.0051	0.0008
	12-0283	0.00014	0.0043	0.0038	0.0076	0.0009	0.0351	0.0021	0.0037	0.0007
	12-0295	ND	0.0036	0.0033	0.0065	0.0008	0.0327	0.0021	0.0042	0.0008
	12-0302	0.00011	0.0037	0.0031	0.0062	0.0008	0.0345	0.0017	0.0035	0.0006
	12-0310	0.00013	0.0038	0.0031	0.0062	0.0011	0.0348	0.0017	0.0034	0.0008
	12-0333	0.00015	0.0040	0.0035	0.0070	0.0014	0.0323	0.0016	0.0035	0.0009
	12-0363	0.00011	0.0038	0.0036	0.0062	0.0013	0.0308	0.0019	0.0032	0.0010
	Mean	**0.000119**	**0.00386**	**0.00336**	**0.00649**	**0.00121**	**0.03388**	**0.00195**	**0.00372**	**0.00081**
	SD	**2.122E-05**	**0.000219**	**0.000224**	**0.000524**	**0.000307**	**0.002015**	**0.000288**	**0.000635**	**0.000136**

ND = No data

Table H-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Male Rats

ORGAN MASS RELATIVE TO BRAIN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
Corn Oil Control	12-0163	0.03153	0.8387	1.6375	0.5299	8.9198	0.4015	1.7070	0.2146
	12-0205	0.04640	0.8371	1.4991	0.6207	7.7770	0.3646	1.6520	0.3348
	12-0206	0.04159	0.8112	1.4808	0.5791	8.0667	0.3633	1.5763	0.2829
	12-0207	0.04322	0.9091	1.6066	0.6169	8.4695	0.4587	1.9317	0.3443
	12-0235	0.04348	0.8797	1.7488	0.6568	9.3451	0.3941	1.6910	0.3511
	12-0254	0.03590	0.8014	1.4737	0.4247	7.1175	0.4713	1.1846	0.3049
	12-0263	0.03686	0.9417	1.5548	0.5894	8.3257	0.4470	1.7704	0.3182
	12-0267	0.03398	1.1364	1.6852	0.7176	9.0115	0.4703	1.8146	0.3188
	12-0284	0.06039	0.9867	1.8997	0.5947	9.4427	0.7052	1.9657	0.3071
	12-0313	0.03342	0.7978	1.3418	0.6768	7.1011	0.3549	1.4886	0.2682
	Mean	**0.040677**	**0.89399**	**1.59279**	**0.60066**	**8.35766**	**0.44310**	**1.67820**	**0.30449**
	SD	**0.0085318**	**0.105959**	**0.159589**	**0.081580**	**0.846434**	**0.102708**	**0.227471**	**0.040725**
31.25 mg/kg	12-0164	0.03531	0.7901	1.7648	0.5568	8.9337	0.4036	1.8659	0.2490
	12-0165	0.03654	0.8320	1.6232	0.6047	9.4466	0.3654	1.8073	0.2867
	12-0225	0.03091	0.7336	1.4919	0.5455	8.2521	0.3827	1.6835	0.2799
	12-0234	0.02937	0.6931	1.3186	0.5766	7.0387	0.3353	1.6579	0.3231
	12-0243	0.03745	0.7499	1.4166	0.5539	6.9621	0.3657	1.6713	0.2737
	12-0245	0.04453	0.8006	1.6665	0.5718	8.2662	0.3856	1.6589	0.3103
	12-0255	0.04799	0.7836	1.3273	0.5324	5.6388	0.4206	1.5220	0.2363
	12-0262	0.03673	0.9884	1.8159	0.6676	9.2743	0.4491	1.7596	0.3928
	12-0324	0.04914	0.8210	1.6150	0.5171	8.2268	0.4504	1.5473	0.3580
	12-0351	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)
	Mean	**0.038662**	**0.799131**	**1.55997**	**0.56958**	**8.00435**	**0.39538**	**1.68597**	**0.30109**
	SD	**0.0070590**	**0.083415**	**0.181448**	**0.044747**	**1.24252**	**0.039146**	**0.112027**	**0.050662**
125 mg/kg	12-0187	0.03735	0.9158	1.5532	0.5735	8.3272	0.4270	1.7655	0.3201
	12-0204	0.03843	0.8693	1.3819	0.6682	8.3800	0.4663	1.8425	0.3383
	12-0223	0.03208	0.8920	1.5545	0.5874	8.2418	0.3764	1.6570	0.2630
	12-0224	0.03525	0.7050	1.5622	0.5636	7.4953	0.3846	1.6542	0.2918
	12-0253	0.04190	0.7864	1.3517	0.5543	6.4719	0.4047	1.8016	0.2785
	12-0275	0.03748	0.7663	1.4224	0.6111	7.4471	0.3426	1.6931	0.2368
	12-0293	0.03332	0.7978	1.3720	0.6508	7.4331	0.4117	1.6061	0.2309
	12-0296	0.03746	0.7597	1.3901	0.5847	6.8136	0.3618	1.8858	0.2325
	12-0345	0.04277	0.7686	1.3800	0.6276	7.2103	0.3773	1.7100	0.2440
	12-0354	0.04871	0.8025	1.3905	0.5590	7.0048	0.3743	1.4853	0.2672
	Mean	**0.038475**	**0.806346**	**1.43586**	**0.59805**	**7.48251**	**0.39266**	**1.71012**	**0.27033**
	SD	**0.0048987**	**0.066028**	**0.085191**	**0.039823**	**0.655775**	**0.035683**	**0.118989**	**0.037256**
500 mg/kg	12-0177	0.04105	0.8178	1.4718	0.3469	8.3496	0.5273	0.7749	0.4407
	12-0189	0.03073	0.7644	1.7702	0.2883	8.6654	0.4366	0.7541	0.2990
	12-0203	0.03819	0.7671	1.4080	0.3270	6.9269	0.4075	0.6716	0.2939
	12-0209	0.03700	0.9070	1.7142	0.3756	8.7877	0.4080	0.7858	0.2512
	12-0236	0.05103	0.9136	1.4328	0.4161	9.0697	0.4328	0.5623	0.4127
	12-0256	0.03879	0.7308	1.3482	0.3841	6.5492	0.3581	0.6741	0.2167
	12-0297	0.05519	0.8401	1.9170	0.5014	8.7354	0.5934	0.7646	0.2976
	12-0314	0.05621	0.8098	1.6262	0.4970	ND	0.4398	0.6525	0.2389
	12-0322	0.04275	0.7613	1.2478	0.3929	5.8439	0.3401	0.6940	0.2743
	12-0341	0.03987	0.8505	1.5449	0.3878	7.6901	0.4191	0.6241	0.2558
	Mean	**0.04308**	**0.816251**	**1.54810**	**0.39169**	**7.84642**	**0.43626**	**0.69582**	**0.29807**
	SD	**0.0083560**	**0.062299**	**0.207629**	**0.067445**	**1.154303**	**0.074751**	**0.073336**	**0.073163**

(f) = Animal died on study.
ND = No data

Table H-8
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Female Rats

ORGAN MASS RELATIVE TO BRAIN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
Corn Oil Control	12-0211	0.0416	0.6911	1.0297	6.2545	0.0609	0.3901	0.1941	0.3005
	12-0220	0.0339	0.6384	0.9222	7.4594	0.0563	0.4699	0.2171	0.2872
	12-0222	0.0364	0.6119	1.0339	6.0964	0.0674	0.3610	0.2371	0.3266
	12-0260	0.0556	0.4742	1.0491	4.9174	0.0866	0.2449	0.0956	0.3976
	12-0289	0.0514	0.6333	0.9790	6.1002	0.0766	0.4134	0.1275	0.3054
	12-0298[a]								
	12-0306	0.0411	0.5450	1.0499	5.4227	0.0695	0.3214	0.1336	0.4149
	12-0309	0.0497	0.5885	1.1160	6.3423	0.0887	0.3564	0.1014	0.3262
	12-0317[b]								
	12-0339	0.0397	0.5095	1.0392	ND	0.1132	ND	0.1566	0.3116
	Mean	**0.04367**	**0.58648**	**1.02738**	**6.08470**	**0.07741**	**0.36532**	**0.15787**	**0.33375**
	SD	**0.007694**	**0.072427**	**0.056595**	**0.794392**	**0.018435**	**0.071189**	**0.053004**	**0.046801**
31.25 mg/kg	12-0168	0.0527	0.6891	1.2606	5.9848	0.0771	0.3291	0.1765	0.4310
	12-0170	0.0541	0.7251	1.1666	8.0229	0.0942	0.4227	0.1229	0.3446
	12-0201[c]	0.0341	0.5631	1.0697	6.6806	0.0646	0.3016	0.0941	0.2472
	12-0221	0.0387	0.5753	0.9781	7.1490	0.0686	0.3219	0.0477	0.3587
	12-0240	0.0285	0.5575	0.9087	5.8571	0.0838	0.2922	0.1547	0.3319
	12-0299[a]								
	12-0300	0.0496	0.5943	1.0868	6.2543	0.0853	0.3090	0.1457	0.4331
	12-0346	0.0507	0.5582	0.9412	5.4738	0.0823	0.3210	0.1960	0.3194
	12-0367	0.0345	0.5390	0.9634	5.1815	0.0679	0.3059	0.1939	0.2967
	12-0369	0.0366	0.6168	1.1088	5.9947	0.0531	0.2903	0.0876	0.3195
	Mean	**0.04218**	**0.60205**	**1.05378**	**6.28874**	**0.07519**	**0.32153**	**0.13545**	**0.34247**
	SD	**0.009612**	**0.064352**	**0.115919**	**0.877311**	**0.0127**	**0.040195**	**0.051361**	**0.059851**
125 mg/kg	12-0169	0.0844	0.5800	1.1932	6.3066	0.0725	0.3843	0.0611	0.5013
	12-0210	0.0322	0.7436	1.0990	6.0080	0.0628	0.3891	0.2187	0.3298
	12-0212	0.0461	0.5683	0.9777	5.8912	0.0876	0.3107	0.0997	0.3117
	12-0227	0.0415	0.6194	0.9397	5.5422	0.0716	0.2577	0.1460	0.3382
	12-0257	0.0449	0.5833	0.8360	5.5279	0.0813	0.3345	0.0703	0.3589
	12-0259	0.0532	0.5093	1.0547	4.4310	0.0908	0.2428	0.1089	0.3919
	12-0261	0.0455	0.6127	1.0545	6.6272	0.0925	0.3298	0.1689	0.3478
	12-0278	0.0488	0.7468	1.2020	6.6295	0.0641	0.2829	0.1532	0.3624
	12-0291	0.0464	0.5751	1.2660	6.3725	0.0756	0.3162	0.0988	0.3968
	12-0327	0.0503	0.5090	1.1386	5.4556	0.0688	0.3180	0.1451	0.4442
	Mean	**0.04932**	**0.60473**	**1.07614**	**5.87917**	**0.07676**	**0.31661**	**0.12706**	**0.37830**
	SD	**0.013571**	**0.082526**	**0.132039**	**0.673204**	**0.010765**	**0.047633**	**0.048267**	**0.057677**
500 mg/kg	12-0166	0.0383	0.5816	0.8955	5.8226	0.0651	0.3270	0.1197	0.3270
	12-0167	0.0346	0.5318	1.0722	6.2962	0.0556	0.3088	0.1173	0.3504
	12-0171[a]								
	12-0198	0.0449	0.6007	1.0279	6.2473	0.0708	0.3340	0.0793	0.2428
	12-0229[a]								
	12-0258	0.0496	0.5961	0.9917	5.5372	0.1012	0.2763	0.1937	0.7154*
	12-0279	0.0385	0.5154	1.0917	4.8094	0.0600	0.1941	0.1063	0.2452
	12-0308[c]	0.0446	0.7321	1.0874	5.6716	0.0946	0.3437	0.1551	0.3269
	12-0336	ND	0.5485	0.9700	5.3048	0.0665	0.2672	0.1379	0.2606
	12-0348	0.0280	0.5642	1.0709	5.4097	0.0589	0.2949	0.1424	0.3055
	Mean	**0.03979**	**0.58380**	**1.02592**	**5.63735**	**0.07159**	**0.29325**	**0.13145**	**0.29407**
	SD	**0.00725**	**0.067032**	**0.069245**	**0.492747**	**0.017017**	**0.048403**	**0.034413**	**0.043972**

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but dam was pregnant.
d= Animal was not fasted prior to necropsy.
* = Identified as an outlier.
ND = No data

Table H-9
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Recovery Male Rats

ORGAN MASS RELATIVE TO BRAIN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Epididymides	Liver	Spleen	Testes	Thymus
Corn Oil Control	12-0174	0.02659	0.9261	1.6332	0.6431	10.1803	0.5611	1.8166	0.2068
	12-0179	0.02607	0.9596	1.6997	0.5677	10.4920	0.6590	1.6278	0.2330
	12-0194	0.03182	0.9738	1.8827	0.5902	10.8583	0.4551	1.5070	0.2132
	12-0208	0.03587	0.7804	1.6325	0.6308	8.5696	0.4064	1.4921	0.2931
	12-0272	0.03444	0.8678	1.7105	0.6076	9.8508	0.4366	1.5030	0.2249
	12-0294	0.02955	0.9515	1.6824	0.5489	8.8038	0.5300	1.6985	0.2101
	12-0304	0.03055	0.8890	1.7785	0.4969	8.8473	0.4955	1.7502	0.2215
	12-0323	0.03311	0.8600	1.6831	0.4352	10.1490	0.4522	1.4934	0.1873
	12-0332	0.03052	0.8914	1.6311	0.5193	7.6346	0.3654	1.5193	0.1508
	12-0365	0.02299	0.8253	1.6533	0.5490	8.7186	0.4524	1.6184	0.1908
	Mean	**0.030151**	**0.89250**	**1.69870**	**0.55886**	**9.41042**	**0.48137**	**1.60263**	**0.21315**
	SD	**0.0039993**	**0.061915**	**0.079020**	**0.063773**	**1.034005**	**0.084271**	**0.119169**	**0.036656**
500 mg/kg	12-0176	0.02721	0.8604	1.6539	0.3439	10.0366	0.5968	0.7614	0.1811
	12-0186	0.02586	0.9269	1.7766	0.4276	9.7468	0.5185	1.1337	0.2211
	12-0215	0.02339	0.8772	1.5042	0.3642	8.6057	0.6470	0.9240	0.2657
	12-0244	0.03424	0.7908	1.6224	0.3837	7.6690	0.4113	1.2254	0.2035
	12-0283	0.03223	0.8697	1.7612	0.1993	8.1280	0.4839	0.8557	0.1729
	12-0295	ND	0.8958	1.7957	0.2180	9.0087	0.5832	1.1504	0.2139
	12-0302	0.02944	0.8377	1.6787	0.2216	9.3778	0.4485	0.9473	0.1558
	12-0310	0.03553	0.8296	1.6370	0.2924	9.2427	0.4546	0.9101	0.2008
	12-0333	0.03684	0.8742	1.7713	0.3536	8.1612	0.4129	0.8909	0.2196
	12-0363	0.02966	0.9449	1.5996	0.3420	8.0162	0.5014	0.8225	0.2525
	Mean	**0.030488**	**0.87071**	**1.68006**	**0.31462**	**8.79927**	**0.50581**	**0.96213**	**0.20869**
	SD	**0.0045721**	**0.045475**	**0.094798**	**0.078107**	**0.802050**	**0.080507**	**0.154481**	**0.033995**

ND = No data

Table H-10
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Absolute Organ Mass (grams)
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Body Weight	Mean	502.3	477.7	473.9	478.6
	S.D.	33.01	43.54	28.04	52.37
	N	10	9	10	10
Adrenals	Mean	0.0860	0.0834	0.0848	0.0912
	S.D.	0.01523	0.01633	0.01175	0.01766
	N	10	9	10	10
Brain	Mean	2.1262	2.155	2.2023	2.1172
	S.D.	0.11397	0.07697	0.05341	0.05304
	N	10	9	10	10
Heart	Mean	1.8930	1.7222	1.7739	1.7282
	S.D.	0.16013	0.18856	0.12335	0.13856
	N	10	9	10	10
Kidneys	Mean	3.3739	3.3602	3.1620	3.2780
	S.D.	0.22721	0.38887	0.19884	0.44738
	N	10	9	10	10
Epididymides	Mean	1.2769	1.2262	1.3166	0.8293[a]
	S.D.	0.18770	0.08860	0.08463	0.14382
	N	10	9	10	10
Liver	Mean	17.7142	17.2361	16.4657	16.5960
	S.D.	1.41119	2.65667	1.31029	2.45914
	N	10	9	10	9
Spleen	Mean	0.9344	0.8532	0.8634	0.9248
	S.D.	0.17329	0.10066	0.06224	0.16826
	N	10	9	10	10
Testes	Mean	3.5535	3.6299	3.7623	1.4750[b]
	S.D.	0.38858	0.21723	0.21267	0.17658
	N	10	9	10	10
Thymus	Mean	0.6457	0.6486	0.5943	0.6314
	S.D.	0.07987	0.11021	0.07325	0.15934
	N	10	9	10	10

[a] = Significantly reduced compared to all other dose groups (p=0.000 for all).
[b] = Significantly reduced compared to all other dose groups (p=0.000 for all).

Table H-11
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Absolute Organ Mass (grams)
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Body Weight	Mean	295.9	303.4	301.7	300.8
	S.D.	33.51	25.12	30.93	24.34
	N	8	9	10	8
Adrenals	Mean	0.0874	0.0811	0.0980	0.0797
	S.D.	0.01468	0.01904	0.02566	0.01402
	N	8	9	10	7
Brain	Mean	2.004625	1.920222[a]	1.9926	1.9853
	S.D.	0.07173	0.05548	0.08395	0.08756
	N	8	9	10	8
Heart	Mean	1.1770	1.1570	1.2071	1.1606
	S.D.	0.16162	0.13698	0.19012	0.16133
	N	8	9	10	8
Kidneys	Mean	2.059375	2.0260	2.1395	2.0411
	S.D.	0.13427	0.25356	0.23383	0.21601
	N	8	9	10	8
Liver	Mean	12.3146	12.1011	11.7056	11.1901
	S.D.	1.81771	1.90478	1.35617	1.08191
	N	7	9	10	8
Ovaries	Mean	0.154375	0.1443	0.1528	0.1421
	S.D.	0.03273	0.02424	0.02135	0.03461
	N	8	9	10	8
Spleen	Mean	0.7389	0.6178[b]	0.6294	0.5824[b]
	S.D.	0.14979	0.08203	0.08766	0.10275
	N	7	9	10	8
Thymus	Mean	0.3175	0.2586	0.2546	0.2606
	S.D.	0.11180	0.09524	0.10010	0.06775
	N	8	9	10	8
Uterus	Mean	0.669375	0.6586	0.7522	0.5863[c]
	S.D.	0.09999	0.12387	0.10591	0.09463
	N	8	9	10	7

[a] = Significantly reduced compared to controls (p=0.024).
[b] = Significantly reduced compared to controls (p=0.035 & 0.008).
[c] = Significantly reduced compared to 125 mg/kg-day group (p=0.027).

Table H-12
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Absolute Organ Mass (grams)
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Body Weight	Mean	597.1	582.0
	S.D.	52.25	52.20
	N	10	10
Adrenals	Mean	0.0677	0.0682
	S.D.	0.00972	0.00905
	N	10	9
Brain	Mean	2.2438	2.2403
	S.D.	0.08356	0.11900
	N	10	10
Heart	Mean	2.0035	1.9500
	S.D.	0.17160	0.13174
	N	10	10
Kidneys	Mean	3.8131	3.7599
	S.D.	0.25580	0.22723
	N	10	10
Epididymides	Mean	1.2568	0.7027[a]
	S.D.	0.17183	0.16969
	N	10	10
Liver	Mean	21.1399	19.7596
	S.D.	2.70018	2.49649
	N	10	10
Spleen	Mean	1.0817	1.1361
	S.D.	0.20864	0.20936
	N	10	10
Testes	Mean	3.5942	2.1521[b]
	S.D.	0.27121	0.33229
	N	10	10
Thymus	Mean	0.4796	0.4669
	S.D.	0.09268	0.07859
	N	10	10

[a] = Significantly reduced compared to controls (p=0.000).
[b] = Significantly reduced compared to controls (p=0.000).

Table H-13
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Organ Mass Relative to Body Mass
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Adrenals	Mean	0.000172	0.000176	0.000180	0.000192
	S.D.	3.188E-05	3.727E-05	2.893E-05	3.799E-05
	N	10	9	10	10
Brain	Mean	0.00425	0.00454	0.00466	0.00447
	S.D.	0.00037413	0.000391617	0.0003002	0.0005031
	N	10	9	10	10
Heart	Mean	0.00377	0.00361	0.00375	0.00363
	S.D.	0.000233	0.000245	0.000212	0.000317
	N	10	9	10	10
Kidneys	Mean	0.00672	0.00703	0.00668	0.00686
	S.D.	0.000333	0.000542	0.000327	0.000653
	N	10	9	10	10
Epididymides	Mean	0.00254	0.00258	0.00279	0.00175[a]
	S.D.	0.000350	0.000169	0.000249	0.000342
	N	10	9	10	10
Liver	Mean	0.03529	0.03599	0.03473	0.03464
	S.D.	0.002190	0.003868	0.001649	0.001945
	N	10	9	10	9
Spleen	Mean	0.00186	0.00179	0.00183	0.00193
	S.D.	0.000331	0.000189	0.000154	0.000216
	N	10	9	10	10
Testes	Mean	0.00709	0.00764	0.00798	0.00310[b]
	S.D.	0.000805	0.000664	0.000826	0.000401
	N	10	9	10	10
Thymus	Mean	0.00129	0.00135	0.00125	0.00131
	S.D.	0.000160	0.000134	0.000139	0.000250
	N	10	9	10	10

[a] = Significantly reduced compared to all other dose groups (p=0.000 for all).

[b] = Significantly reduced compared to all other dose groups (p=0.000 for all).

Table H-14
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Organ Mass Relative to Body Mass
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Adrenals	Mean	0.00030	0.00027	0.00033	0.00026
	S.D.	0.000068	0.000063	0.000104	0.000052
	N	8	9	10	7
Brain	Mean	0.00684	0.00636	0.00666	0.00663
	S.D.	0.000652	0.000420	0.000688	0.000421
	N	8	9	10	8
Heart	Mean	0.00397	0.00381	0.00399	0.00386
	S.D.	0.000206	0.000281	0.000325	0.000406
	N	8	9	10	8
Kidneys	Mean	0.00704	0.00667	0.00715	0.00680
	S.D.	0.000885	0.000557	0.001004	0.000655
	N	8	9	10	8
Liver	Mean	0.04064	0.03976	0.03881	0.03719
	S.D.	0.003719	0.004128	0.002497	0.001526
	N	7	9	10	8
Ovaries	Mean	0.00054	0.00048	0.00052	0.00047
	S.D.	0.000169	0.000083	0.000113	0.000114
	N	8	9	10	8
Spleen	Mean	0.00243	0.00204[a]	0.00209	0.00193[a]
	S.D.	0.000362	0.000216	0.000255	0.000236
	N	7	9	10	8
Thymus	Mean	0.00106	0.00087	0.00083	0.00087
	S.D.	0.000295	0.000361	0.000276	0.000235
	N	8	9	10	8
Uterus	Mean	0.00230	0.00217	0.00253	0.00193
	S.D.	0.000510	0.000373	0.000517	0.000227
	N	8	9	10	7

[a] = Significantly reduced compared to controls (p=0.031 & 0.005).

Table H-15
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Organ Mass Relative to Body Mass
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Adrenals	Mean	0.000114	0.000119
	S.D.	1.838E-05	2.122E-05
	N	10	9
Brain	Mean	0.00378	0.00386
	S.D.	0.000324868	0.000219369
	N	10	10
Heart	Mean	0.00337	0.00336
	S.D.	0.000306	0.000224
	N	10	10
Kidneys	Mean	0.00641	0.00649
	S.D.	0.000441	0.000524
	N	10	10
Epididymides	Mean	0.00211	0.00121[a]
	S.D.	0.000268	0.000307
	N	10	10
Liver	Mean	0.03535	0.03388
	S.D.	0.002474	0.002015
	N	10	10
Spleen	Mean	0.00181	0.00195
	S.D.	0.000255	0.000288
	N	10	10
Testes	Mean	0.00605	0.00372[b]
	S.D.	0.000528	0.000635
	N	10	10
Thymus	Mean	0.00080	0.00081
	S.D.	0.000127053	0.000136063
	N	10	10

[a] = Significantly reduced compared to controls (p=0.000).
[b] = Significantly reduced compared to controls (p=0.000).

Table H-16
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Organ Mass Relative to Brain Mass
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Adrenals	Mean	0.040677	0.038662	0.038475	0.043080
	S.D.	0.0085318	0.0070590	0.0048987	0.0083560
	N	10	9	10	10
Heart	Mean	0.89399	0.79913	0.80635	0.81625
	S.D.	0.105959	0.083415	0.066028	0.062299
	N	10	9	10	10
Kidneys	Mean	1.59279	1.55997	1.43586	1.54810
	S.D.	0.159589	0.181448	0.085191	0.207629
	N	10	9	10	10
Epididymides	Mean	0.60066	0.56958	0.59805	0.39169[a]
	S.D.	0.081580	0.044747	0.039823	0.067445
	N	10	9	10	10
Liver	Mean	8.35766	8.00435	7.48251	7.84642
	S.D.	0.846434	1.242520	0.655775	1.154303
	N	10	9	10	9
Spleen	Mean	0.44310	0.39538	0.39266	0.43626
	S.D.	0.102708	0.039146	0.035683	0.074751
	N	10	9	10	10
Testes	Mean	1.67820	1.68597	1.71012	0.69582[b]
	S.D.	0.227471	0.112027	0.118989	0.073336
	N	10	9	10	10
Thymus	Mean	0.30449	0.30109	0.27033	0.29807
	S.D.	0.040725	0.050662	0.037256	0.073163
	N	10	9	10	10

[a] = Significantly reduced compared to all other dose groups (p=0.000 for all).
[b] = Significantly reduced compared to all other dose groups (p=0.000 for all).

Table H-17
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Organ Mass Relative to Brain Mass
Female Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
Adrenals	Mean	0.04367	0.04218	0.04932	0.03979
	S.D.	0.007694	0.009612	0.0135711	0.0072499
	N	8	9	10	7
Heart	Mean	0.58648	0.60205	0.60473	0.58380
	S.D.	0.072427	0.064352	0.0825256	0.0670317
	N	8	9	10	8
Kidneys	Mean	1.02738	1.05378	1.07614	1.02592
	S.D.	0.056595	0.115919	0.132039	0.0692448
	N	8	9	10	8
Liver	Mean	6.08470	6.28874	5.87917	5.63735
	S.D.	0.794392	0.877311	0.6732044	0.492747
	N	7	9	10	8
Ovaries	Mean	0.07741	0.07519	0.07676	0.07159
	S.D.	0.018435	0.012700	0.0107646	0.0170166
	N	8	9	10	8
Spleen	Mean	0.36532	0.32153	0.31661	0.29325
	S.D.	0.071189	0.040195	0.0476333	0.0484033
	N	7	9	10	8
Thymus	Mean	0.15787	0.13545	0.12706	0.13145
	S.D.	0.053004	0.051361	0.0482673	0.0344125
	N	8	9	10	8
Uterus	Mean	0.33375	0.34247	0.37830	0.29407[a]
	S.D.	0.046801	0.059851	0.0576766	0.043972
	N	8	9	10	7

[a] = Significantly reduced compared to 125 mg/kg-day group (p=0.016).

Table H-18
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

Summary Organ Mass Relative to Brain Mass
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Adrenals	Mean	0.030151	0.030488
	S.D.	0.0039993	0.0045721
	N	10	9
Heart	Mean	0.89250	0.87071
	S.D.	0.061915	0.045475
	N	10	10
Kidneys	Mean	1.69870	1.68006
	S.D.	0.079020	0.094798
	N	10	10
Epididymides	Mean	0.55886	0.31462[a]
	S.D.	0.063773	0.078107
	N	10	10
Liver	Mean	9.41042	8.79927
	S.D.	1.034005	0.802050
	N	10	10
Spleen	Mean	0.48137	0.50581
	S.D.	0.084271	0.080507
	N	10	10
Testes	Mean	1.60263	0.96213[b]
	S.D.	0.119169	0.154481
	N	10	10
Thymus	Mean	0.21315	0.20869
	S.D.	0.036656	0.033995
	N	10	10

[a] = Significantly reduced compared to controls (p=0.000).
[b] = Significantly reduced compared to controls (p=0.000).

Table H-19
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Non-Pregnant Female Rats

ABSOLUTE ORGAN MASS (grams)

Group	Animal ID	Body Weight*	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus	Implantation Sites	Corpora Lutea
Control	12-0298[a,d]	280	0.065	1.931	0.978	1.842	8.793	0.180	0.509	0.476	0.585	n/a	n/a
	12-0317[b]	330	0.063	2.046	1.052	1.892	9.424	0.148	0.527	0.394	0.492	n/a	n/a
	Mean	**305.0**	**0.0640**	**1.9885**	**1.0150**	**1.867**	**9.1085**	**0.164**	**0.5180**	**0.4350**	**0.5385**		
	SD	**35.36**	**0.00141**	**0.08132**	**0.05233**	**0.03536**	**0.44618**	**0.02263**	**0.01273**	**0.05798**	**0.06576**		
31.25 mg/kg	12-0299[a]	330	0.076	1.934	1.159	2.292	9.806	0.184	0.754	0.528	0.619	n/a	n/a
	Mean	**330.0**	**0.0760**	**1.934**	**1.1590**	**2.2920**	**9.8060**	**0.1840**	**0.7540**	**0.5280**	**0.6190**		
	SD												
500 mg/kg	12-0171[a]	304	0.066	1.916	1.025	1.843	9.692	0.129	0.605	0.307	0.413	n/a	n/a
	12-0229[a]	258	0.075	1.956	0.984	1.856	7.504	0.119	0.510	0.350	0.851	n/a	n/a
	Mean	**281.0**	**0.0705**	**1.9360**	**1.0045**	**1.8495**	**8.5980**	**0.1240**	**0.5575**	**0.3285**	**0.6320**		
	SD	**32.53**	**0.00636**	**0.02828**	**0.02899**	**0.00919**	**1.54715**	**0.00707**	**0.06718**	**0.03041**	**0.30971**		

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
d= Animal was not fasted prior to necropsy.

Table H-20
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Non-Pregnant Female Rats

ORGAN MASS RELATIVE TO BODY MASS

Group	Animal ID	Adrenals	Brain	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
Corn Oil Control	12-0298[a]	0.0002	0.0069	0.0035	0.0066	0.0314	0.0006	0.0018	0.0017	0.0021
	12-0317[b]	0.0002	0.0062	0.0032	0.0057	0.0286	0.0004	0.0016	0.0012	0.0015
	Mean	**0.00021**	**0.00655**	**0.00334**	**0.00616**	**0.02998**	**0.00055**	**0.00171**	**0.00145**	**0.00179**
	SD	**0.000029**	**0.000492**	**0.000216**	**0.000598**	**0.002012**	**0.000137**	**0.000156**	**0.000358**	**0.000423**
31.25 mg/kg	12-0299[a]	0.0002	0.0059	0.0035	0.0069	0.0297	0.0006	0.0023	0.0016	0.0019
	Mean	**0.00023**	**0.00586**	**0.00351**	**0.00695**	**0.02972**	**0.00056**	**0.00228**	**0.00160**	**0.00188**
	SD									
500 mg/kg	12-0171[a]	0.0002	0.0063	0.0034	0.0061	0.0319	0.0004	0.0020	0.0010	0.0014
	12-0229[a]	0.0003	0.0076	0.0038	0.0072	0.0291	0.0005	0.0020	0.0014	0.0033
	Mean	**0.00025**	**0.00694**	**0.00359**	**0.00663**	**0.03048**	**0.00044**	**0.00198**	**0.00118**	**0.00233**
	SD	**0.000052**	**0.000904**	**0.000313**	**0.000800**	**0.001977**	**0.000026**	**0.000009**	**0.000245**	**0.001372**

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.

Table H-21
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Organ Mass
Non-Pregnant Female Rats

ORGAN MASS RELATIVE TO BRAIN MASS

Group	Animal ID	Adrenals	Heart	Kidneys	Liver	Ovaries	Spleen	Thymus	Uterus
Corn Oil Control	12-0298[a]	0.0337	0.5065	0.9539	4.5536	0.0932	0.2636	0.2465	0.3030
	12-0317[b]	0.0308	0.5142	0.9247	4.6061	0.0723	0.2576	0.1926	0.2405
	Mean	**0.04138**	**0.57125**	**1.00977**	**5.75028**	**0.07848**	**0.34204**	**0.17020**	**0.32134**
	SD	**0.008354**	**0.071515**	**0.062587**	**0.955934**	**0.017137**	**0.077046**	**0.05498**	**0.051036**
31.25 mg/kg	12-0299[a]	0.0393	0.5993	1.1851	5.0703	0.0951	0.3899	0.2730	0.3201
	Mean	**0.04189**	**0.60177**	**1.06691**	**6.16690**	**0.07718**	**0.32837**	**0.14920**	**0.34022**
	SD	**0.009108**	**0.060678**	**0.116914**	**0.912475**	**0.013534**	**0.043624**	**0.065094**	**0.056871**
500 mg/kg	12-0171[a]	0.0344	0.5350	0.9619	5.0585	0.0673	0.3158	0.1602	0.2156
	12-0229[a]	0.0383	0.5031	0.9489	3.8364	0.0608	0.2607	0.1789	0.4351
	Mean	**0.03903**	**0.57085**	**1.01181**	**5.39937**	**0.07009**	**0.29225**	**0.13908**	**0.34245**
	SD	**0.006528**	**0.065555**	**0.067994**	**0.723554**	**0.015413**	**0.044664**	**0.034626**	**0.145965**

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.

Appendix I

Individual and Summary of Sperm Analysis Data

Table I-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Sperm Analysis
Male Rats

Dose	Animal/Sample ID	Sperm Count (million)*	Sample Weight (grams)	Sperm Count/ Sample (million/gram)*
Corn Oil Control	12-0163	35.05	0.209	167.7000
	12-0205	33.25	0.216	153.9500
	12-0206	30.95	0.179	172.9000
	12-0207	35.55	0.225	158.0500
	12-0235	38.40	0.275	139.5500
	12-0254	37.70	0.243	155.2500
	12-0263	40.15	0.226	177.7000
	12-0267	54.75	0.249	219.8500
	12-0284	31.55	0.206	153.3500
	12-0313	39.35	0.217	181.5500
	Mean	**37.67**	**0.2245**	**167.98500**
	SD	**6.79**	**0.02641**	**22.294008**
31.25 mg/kg	12-0164	41.05	0.222	184.9500
	12-0165	39.75	0.258	154.2000
	12-0225	37.10	0.208	178.3000
	12-0234	30.80	0.216	142.7000
	12-0243	34.45	0.203	169.5000
	12-0245	23.65	0.198	119.5000
	12-0255	23.15	0.197	117.5500
	12-0262	49.10	0.242	202.9500
	12-0324	22.35	0.200	111.9000
	12-0351	(f)	(f)	(f)
	Mean	**33.5**	**0.216**	**153.50556**
	SD	**9.28**	**0.02136**	**32.790123**
125 mg/kg	12-0187	34.25	0.213	160.9000
	12-0204	48.00	0.270	177.6500
	12-0223	27.85	0.222	125.6000
	12-0224	27.10	0.201	134.9000
	12-0253	23.80	0.220	108.1500
	12-0275	47.20	0.230	205.2000
	12-0293	32.60	0.238	137.0500
	12-0296	7.80	0.178	43.8500
	12-0345	42.85	0.231	185.5000
	12-0354	36.60	0.196	186.6500
	Mean	**32.805**	**0.2199**	**146.54500**
	SD	**12.11**	**0.02538**	**47.638444**
500 mg/kg	12-0177	0.90	0.115	7.8000
	12-0189	3.20	0.103	31.0500
	12-0203	0.90	0.082	10.9500
	12-0209	0.30	0.121	2.1000
	12-0236	2.55	0.116	22.0500
	12-0256	0.40	0.099	3.9000
	12-0297	2.05	0.114	17.9500
	12-0314	1.65	0.120	16.3000
	12-0322	0.15	0.098	1.3000
	12-0341	0.15	0.102	1.2500
	Mean	**1.2**	**0.1070**	**11.46500**
	SD	**1.08**	**0.01234**	**10.150809**

(f) = Animal died on study
* = Value represents average of 2 readings for each animal.

Table I-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Sperm Analysis
Recovery Male Rats

Dose	Animal/Sample ID	Sperm Count (million)*	Sample Weight (grams)	Sperm Count/ Sample (million/gram)*
Corn Oil Control	12-0174	34.40	0.263	130.8000
	12-0179	59.00	0.310	190.2000
	12-0194	38.00	0.271	140.2000
	12-0208	36.70	0.282	130.1500
	12-0272	50.10	0.305	164.3500
	12-0294	36.45	0.323	112.8500
	12-0304	16.65	0.318	52.3000
	12-0323	10.60	0.247	42.9500
	12-0332	25.35	0.288	87.9500
	12-0365	11.50	0.320	36.0000
	Mean	**31.875**	**0.2927**	**108.77500**
	SD	**15.98**	**0.02652**	**52.617040**
500 mg/kg	12-0176	5.85	0.121	48.6000
	12-0186	5.90	0.142	41.4000
	12-0215	2.95	0.156	18.8500
	12-0244	3.85	0.133	28.8500
	12-0283	8.85	0.140	63.0500
	12-0295	3.45	0.131	26.3500
	12-0302	1.95	0.112	17.1000
	12-0310	4.85	0.128	38.0000
	12-0333	1.80	0.126	14.2000
	12-0363	2.05	0.138	14.8500
	Mean	**4.2**	**0.1327**	**31.12500**
	SD	**2.24**	**0.01223**	**16.343895**

(f) = Animal died on study
* = Value represents average of 2 readings for each animal.

Table I-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Sperm Analysis
Male Rats

Period		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	NTO in Corn Oil 125 mg/kg	NTO in Corn Oil 500 mg/kg
Sperm Count (million/gram sample)	Mean	167.98500	153.50556	146.54500	11.46500[a]
	S.D.	22.294008	32.790123	47.638444	10.150809
	N	10	9	10	10

Summary of Sperm Analysis
Recovery Male Rats

Period		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Sperm Count (million/gram sample)	Mean	108.77500	31.12500[b]
	S.D.	52.617040	16.343895
	N	10	10

[a] = Significantly reduced compared to all other dose groups (p=0.000 for all).
[b] = Significantly reduced compared to controls (p=0.000).

Appendix J

Individual and Summary of Clinical Chemistry Data

Table J-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Clinical Chemistry Results
Male Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	NOTES
Control	12-0163	3.3	211	60	82	13	11.5	69	0.5	3.2	314	499	11.8	0.5	6.5	150	10.3	104	
	12-0205	3.3	130	60	97	18	11.1	57	0.6	3.3	155	733	11.7	0.9	6.6	152	11.0	105	
	12-0206	3.3	227	53	79	14	11.5	75	0.6	3.6	152	88	12.0	0.4	6.8	155	8.7	102	
	12-0207	3.2	162	55	106	21	11.2	39	0.6	3.5	143	845	11.6	0.7	6.7	150	11.2	105	
	12-0235	3.9	182	64	74	ND	11.9	74	0.5	3.9	273	ND	12.8	1.3	7.8	ND	ND	ND	SS
	12-0254	3.5	313	55	88	10	11.1	58	0.4	3.1	202	172	9.8	0.6	6.6	152	8.9	106	
	12-0263	3.5	255	60	92	13	11.8	82	0.5	3.3	204	672	11.1	0.6	6.8	153	9.7	104	
	12-0267	3.6	224	54	111	14	12.0	70	0.5	3.4	226	975	12.0	0.9	7.0	153	9.7	103	1:1 dilution AST
	12-0284	3.2	147	51	82	14	10.8	43	0.6	3.6	142	1064	11.0	1.0	6.8	150	10.6	105	
	12-0313	3.4	240	40	72	10	11.9	58	0.4	3.2	240	391	10.6	0.5	6.6	ND	ND	ND	
	Mean	**3.42**	**209.1**	**55.2**	**88.3**	**14.1**	**11.5**	**62.5**	**0.52**	**3.41**	**205.1**	**604.3**	**11.44**	**0.74**	**6.82**	**151.9**	**10.01**	**104.3**	
	SD	**0.215**	**55.25**	**6.68**	**13.14**	**3.52**	**0.42**	**14.03**	**0.079**	**0.242**	**58.98**	**342.39**	**0.844**	**0.280**	**0.374**	**1.81**	**0.923**	**1.28**	
31.25 mg/kg	12-0164	3.3	146	49	59	12	11.7	55	0.6	3.2	262	461	12.7	0.5	6.6	151	11.1	104	
	12-0165	3.2	218	45	65	11	11.0	74	0.3	3.1	180	472	9.1	0.5	6.4	150	6.8	103	
	12-0225	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	SS
	12-0234	3.1	229	63	82	17	11.0	74	0.4	3.4	134	724	11.5	0.8	6.6	149	11.0	103	
	12-0243	3.5	316	51	81	12	11.6	80	0.6	3.1	295	239	11.2	0.4	6.6	148	9.5	102	
	12-0245	3.4	284	51	88	12	11.6	68	0.5	3.3	227	798	11.7	0.9	6.6	151	10.3	102	
	12-0255	3.4	188	69	94	14	11.6	47	0.5	3.3	182	789	11.4	0.9	6.8	153	9.4	102	
	12-0262	3.3	262	53	72	14	11.8	96	0.6	2.9	237	431	12.4	0.5	6.2	152	9.4	101	1:1 dilution AST
	12-0324	3.4	202	47	104	11	11.6	80	0.6	3.6	168	694	11.5	0.7	6.9	151	10.0	104	
	12-0351	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	
	Mean	**3.33**	**230.6**	**53.5**	**80.6**	**12.9**	**11.5**	**71.8**	**0.51**	**3.24**	**210.6**	**576.0**	**11.44**	**0.65**	**6.59**	**150.6**	**9.69**	**102.6**	
	SD	**0.128**	**54.88**	**8.26**	**14.97**	**2.03**	**0.31**	**15.31**	**0.113**	**0.213**	**53.68**	**203.23**	**1.077**	**0.200**	**0.217**	**1.60**	**1.348**	**1.06**	
125 mg/kg	12-0187	3.5	211	48	70	14	10.9	65	0.5	3.3	130	401	11.0	0.3	6.7	153	8.9	102	1:1 dilution LDH
	12-0204	3.3	161	50	79	13	11.4	53	0.6	3.1	217	383	12.0	0.4	6.4	153	9.8	105	
	12-0223	3.3	223	52	67	16	11.4	88	0.5	5.5	217	691	13.2	0.8	8.8	151	9.8	102	
	12-0224	3.2	206	49	73	13	11.1	75	0.7	2.9	158	459	11.0	0.6	6.1	150	9.6	103	
	12-0253	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
	12-0275	3.5	242	48	124	11	11.0	58	0.4	3.1	146	650	12.5	0.6	6.6	151	10.3	105	1:1 dilution AST
	12-0293	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	SS
	12-0296	3.4	277	60	76	11	11.9	70	0.6	2.9	274	540	13.5	0.4	6.3	ND	ND	ND	
	12-0345	3.6	312	52	87	10	12.5	78	0.6	3.1	302	495	13.9	0.7	6.7	ND	ND	ND	
	12-0354	3.6	223	56	87	15	11.7	74	0.6	3.0	232	523	11.6	0.5	6.6	153	9.7	105	
	Mean	**3.43**	**231.9**	**51.9**	**82.9**	**12.9**	**11.5**	**70.1**	**0.56**	**3.36**	**209.5**	**517.8**	**12.34**	**0.54**	**6.78**	**151.8**	**9.68**	**103.7**	
	SD	**0.149**	**46.05**	**4.22**	**18.14**	**2.10**	**0.53**	**11.26**	**0.092**	**0.873**	**61.34**	**109.40**	**1.121**	**0.169**	**0.845**	**1.33**	**0.454**	**1.51**	
500 mg/kg	12-0177	3.8	182	71	117	14	11.9	86	0.4	3.4	238	197	12.8	0.6	7.2	153	9.5	104	
	12-0189	3.4	171	51	81	15	11.0	76	0.5	3.3	255	793	12.1	0.9	6.8	151	10.6	102	
	12-0203	3.3	220	55	92	14	11.6	69	0.5	3.6	217	730	11.7	0.8	6.9	154	8.6	105	
	12-0209	3.0	188	66	242	10	11.0	51	0.4	3.4	110	634	10.8	0.6	6.4	154	8.9	105	1:2 dilution AST
	12-0236	3.4	181	64	99	14	11.7	58	0.4	3.7	218	1401	13.3	1.4	7.1	ND	ND	ND	
	12-0256	3.6	135	45	77	14	11.2	42	0.4	3.5	200	1542	13.5	1.7	7.0	ND	ND	ND	
	12-0297	3.4	190	60	80	12	12.1	66	0.6	3.4	222	701	12.0	0.9	6.8	151	10.3	104	
	12-0314	3.7	193	50	59	16	12.1	54	0.5	3.6	258	694	13.0	0.8	7.3	ND	ND	ND	
	12-0322	3.4	335	56	122	11	11.6	70	0.5	3.3	216	371	13.5	0.7	6.7	ND	ND	ND	
	12-0341	3.6	204	62	76	15	11.7	76	0.4	3.2	229	493	12.9	0.6	6.8	ND	ND	ND	
	Mean	**3.46**	**199.9**	**58.0**	**104.5**	**13.5**	**11.6**	**64.8**	**0.46**	**3.44**	**216.3**	**755.6**	**12.56**	**0.90**	**6.90**	**152.6**	**9.58**	**104.0**	
	SD	**0.227**	**52.37**	**8.06**	**52.01**	**1.90**	**0.41**	**13.41**	**0.070**	**0.158**	**41.46**	**419.99**	**0.885**	**0.368**	**0.262**	**1.52**	**0.864**	**1.22**	

(f) = Animal died on study
ND = No data
SS = Short sample

Table J-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Clinical Chemistry Results
Female Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	NOTES
Control	12-0211	3.3	166	92	110	20	11.6	69	0.8	3.4	105	389	10.0	0.4	6.7	152	9.2	104	slightly lipemic
	12-0220	2.8	221	109	143	14	11.7	78	0.3	2.9	159	323	12.1	0.4	5.6	147	10.0	108	lipemic
	12-0222	3.3	132	75	91	16	11.3	103	0.7	3.1	107	551	11.1	0.4	6.5	150	8.6	108	slightly hemolyzed
	12-0260	3.0	105	67	132	3	12.1	47	0.4	2.8	166	737	13.2	0.3	5.7	151	7.6	105	
	12-0289	3.6	51	88	104	20	11.2	79	0.4	3.2	91	2708	11.3	1.7	6.8	149	9.8	105	1:2 dilution LDH, hemolyzed
	12-0298[a]																		
	12-0306	3.0	303	112	91	12	12.3	71	0.5	2.9	230	388	12.8	0.1	5.9	148	9.0	104	
	12-0309	3.1	175	85	99	17	11.6	80	0.4	3.1	113	391	8.5	< 0.1	6.3	152	6.5	106	
	12-0317[b]																		
	12-0339	3.3	91	82	70	6	13.2	66	0.5	2.9	254	251	14.2	0.3	6.2	149	11.2	103	
	Mean	**3.18**	**155.5**	**88.8**	**105.0**	**13.5**	**11.9**	**74.1**	**0.50**	**3.04**	**153.1**	**717.3**	**11.65**	**0.46**	**6.21**	**149.8**	**8.99**	**105.4**	
	SD	**0.249**	**79.93**	**15.51**	**23.46**	**6.23**	**0.65**	**15.79**	**0.169**	**0.200**	**61.18**	**818.23**	**1.832**	**0.515**	**0.449**	**1.83**	**1.458**	**1.85**	
31.25 mg/kg	12-0168	3.4	100	63	67	14	12.2	62	0.7	2.4	170	340	11.4	< 0.1	5.8	147	9.4	103	
	12-0170	3.4	125	87	69	12	12.9	81	0.5	2.7	276	220	12.5	0.3	6.1	149	10.0	104	
	12-0201[c]	3.5	130	88	94	19	12.1	72	0.4	2.8	213	741	15.4	0.8	6.3	149	10.6	105	hemolyzed, lipemic
	12-0221	2.6	249	112	126	17	11.2	92	0.4	2.7	145	315	11.0	0.3	5.3	149	8.0	105	
	12-0240	3.5	248	101	95	21	12.8	127	0.6	3.3	92	273	13.3	0.3	6.7	148	10.3	102	
	12-0299[a]																		
	12-0300	3.0	91	64	72	10	11.5	86	0.4	2.8	151	425	10.2	0.3	5.8	150	8.3	106	slightly lipemic
	12-0346	3.5	92	87	141	25	11.9	64	0.5	3.2	119	700	13.6	0.9	6.7	149	11.6	108	slightly lipemic and hemolyzed
	12-0367	2.7	150	226	126	16	11.6	60	0.4	2.5	134	546	10.7	0.5	5.2	148	8.9	103	slightly lipemic and hemolyzed
	12-0369	2.9	185	126	108	17	11.6	62	0.6	2.7	158	326	9.9	< 0.1	5.6	148	9.2	105	
	Mean	**3.17**	**152.2**	**106.0**	**99.8**	**16.8**	**12.0**	**78.4**	**0.50**	**2.79**	**162.0**	**431.8**	**12.00**	**0.40**	**5.94**	**148.6**	**9.59**	**104.6**	
	SD	**0.367**	**62.20**	**49.40**	**27.37**	**4.58**	**0.58**	**21.61**	**0.112**	**0.293**	**54.34**	**188.40**	**1.829**	**0.283**	**0.550**	**0.88**	**1.150**	**1.81**	
125 mg/kg	12-0169	3.8	100	65	92	11	12.5	71	0.6	2.8	188	795	12.1	0.2	6.6	150	10.8	104	
	12-0210	3.7	107	46	82	20	11.4	78	0.6	3.0	109	337	11.4	0.4	6.7	152	9.8	107	slightly lipemic
	12-0212	3.1	154	87	118	16	11.9	77	0.6	3.1	118	587	11.1	0.5	6.2	155	9.1	105	slightly lipemic
	12-0227	2.8	182	75	88	9	11.7	75	0.4	2.7	102	334	11.6	< 0.1	5.5	153	8.0	107	lipemic
	12-0257	3.1	193	91	103	18	11.4	63	0.5	2.9	96	506	10.7	0.2	6.1	152	8.3	106	lipemic
	12-0259	3.6	130	87	95	4	12.4	45	0.4	2.8	195	504	10.5	0.2	6.4	147	9.0	99	
	12-0261	3.4	135	132	206	23	12.2	74	0.5	2.6	105	2979	13.1	0.7	6.0	150	10.6	107	1:1 dilution LDH, slightly lipemic and hemolyzed
	12-0278	3.3	98	95	95	14	11.8	63	0.8	3.0	138	315	11.5	< 0.1	6.3	149	9.0	100	slightly lipemic
	12-0291	2.4	120	117	103	14	10.8	52	0.6	2.7	157	282	10.7	< 0.1	5.1	149	9.1	105	
	12-0327	3.1	131	85	87	14	11.6	40	0.6	2.8	167	296	9.8	0.1	5.9	150	10.7	106	
	Mean	**3.23**	**135.0**	**88.0**	**106.9**	**14.3**	**11.8**	**63.8**	**0.56**	**2.84**	**137.5**	**693.5**	**11.25**	**0.26**	**6.08**	**150.7**	**9.44**	**104.6**	
	SD	**0.427**	**32.62**	**24.29**	**36.29**	**5.48**	**0.51**	**13.81**	**0.117**	**0.158**	**37.03**	**819.43**	**0.922**	**0.207**	**0.489**	**2.31**	**0.995**	**2.88**	
500 mg/kg	12-0166	3.5	93	67	72	16	12.4	81	0.5	3.2	127	371	15.5	0.2	6.7	151	9.6	108	
	12-0167	3.5	109	60	66	18	12.3	75	0.5	2.9	123	244	12.5	0.4	6.4	149	12.3	106	
	12-0171[a]																		
	12-0198	3.5	91	79	95	20	12.4	75	0.7	2.8	184	374	14.6	0.2	6.3	154	9.2	107	slightly lipemic
	12-0229[a]																		
	12-0258	3.5	96	73	79	8	12.1	56	0.5	2.8	155	340	12.4	0.2	6.3	150	9.9	106	slightly lipemic
	12-0279	3.2	178	124	118	8	12.0	68	0.4	2.6	163	443	13.4	0.1	5.8	147	6.6	102	lipemic
	12-0308[c]	3.2	93	100	110	15	11.7	60	0.7	2.8	108	503	12.8	0.4	6.0	148	12.3	108	lipemic
	12-0336	3.4	97	115	114	14	12.4	81	0.5	2.9	144	654	13.0	< 0.1	6.3	146	12.9	107	hemolyzed
	12-0348	3.3	270	96	81	17	12.1	64	0.5	3.1	148	421	12.8	0.5	6.3	ND	ND	ND	lipemic
	Mean	**3.39**	**128.4**	**89.3**	**91.9**	**14.5**	**12.2**	**70.0**	**0.54**	**2.89**	**144.0**	**418.8**	**13.38**	**0.26**	**6.26**	**149.3**	**10.40**	**106.3**	
	SD	**0.136**	**64.17**	**23.15**	**20.21**	**4.41**	**0.25**	**9.47**	**0.106**	**0.189**	**24.27**	**121.92**	**1.104**	**0.151**	**0.267**	**2.69**	**2.245**	**2.06**	

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.
(f) = Animal died on study
ND = No data
SS = Short sample

Table J-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Clinical Chemistry Results
Recovery Male Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	NOTES
Control	12-0174	3.4	118	40	51	17	11.3	131	0.5	3.2	242	296	10.7	0.5	6.6	153	7.5	104	
	12-0179	3.7	98	29	64	17	11.9	99	0.5	3.2	207	422	11.8	0.8	7.0	151	10.6	106	
	12-0194	3.5	111	38	65	14	11.5	73	0.7	2.8	306	278	11.7	0.3	6.3	149	11.3	103	
	12-0208	3.6	101	29	75	21	11.9	86	0.7	3.3	214	435	11.5	0.5	6.9	153	8.6	104	
	12-0272	3.3	152	44	90	17	11.8	83	0.6	3.4	320	545	11.2	0.5	6.6	150	9.4	103	
	12-0294	3.5	109	39	80	15	11.5	79	0.5	3.5	226	575	10.8	0.7	7.0	151	8.3	104	slightly hemolyzed
	12-0304	3.5	101	45	69	18	12.5	67	0.6	3.2	279	340	11.0	0.3	6.7	152	8.3	106	
	12-0323	3.5	158	48	100	19	12.5	116	0.5	3.5	195	441	9.3	0.4	7.0	152	7.6	105	
	12-0332	3.6	175	52	60	19	12.8	113	0.8	3.1	261	354	12.0	0.3	6.8	151	11.1	107	
	12-0365	3.7	96	46	64	13	12.7	114	0.7	3.2	281	369	10.8	0.4	6.9	151	9.1	102	
	Mean	**3.53**	**121.9**	**41.0**	**71.8**	**17.0**	**12.0**	**96.1**	**0.61**	**3.24**	**253.1**	**405.5**	**11.08**	**0.47**	**6.78**	**151.3**	**9.18**	**104.4**	
	SD	**0.125**	**28.76**	**7.62**	**14.73**	**2.45**	**0.54**	**21.54**	**0.110**	**0.207**	**43.04**	**98.38**	**0.776**	**0.170**	**0.230**	**1.25**	**1.393**	**1.58**	
500 mg/kg	12-0176	3.7	121	24	77	18	11.9	123	0.5	3.4	290	388	11.5	0.6	7.1	153	9.1	105	
	12-0186	3.5	110	27	63	14	11.4	109	0.5	3.1	208	83	10.6	0.4	6.6	150	9.3	104	
	12-0215	3.5	156	45	68	13	11.8	104	0.6	3.1	285	265	11.7	0.5	6.5	153	8.4	106	
	12-0244	3.2	123	32	42	17	11.5	75	0.7	3.1	207	330	9.9	0.3	6.3	152	9.3	105	
	12-0283	3.6	103	52	105	35	11.3	94	0.6	3.5	165	784	12.8	0.7	7.1	152	9.8	105	slightly hemolyzed
	12-0295	3.2	91	44	55	20	11.5	68	0.4	3.5	196	540	9.8	0.3	6.6	152	8.2	104	
	12-0302	3.4	115	41	59	16	11.4	86	0.5	3.8	233	374	10.4	0.2	7.2	147	11.0	100	
	12-0310	3.4	114	45	58	18	12.1	77	0.5	3.2	208	310	10.3	< 0.1	6.5	149	8.9	104	
	12-0333	3.7	151	47	51	15	12.3	93	0.7	3.2	245	288	11.5	0.4	6.9	149	10.0	103	
	12-0363	3.4	85	55	71	14	12.2	93	0.6	3.3	191	372	10.5	0.4	6.7	152	8.6	104	
	Mean	**3.46**	**116.9**	**41.2**	**64.9**	**18.0**	**11.7**	**92.2**	**0.56**	**3.32**	**222.8**	**373.4**	**10.90**	**0.39**	**6.75**	**150.9**	**9.26**	**104.0**	
	SD	**0.178**	**22.82**	**10.33**	**17.33**	**6.36**	**0.37**	**16.75**	**0.097**	**0.230**	**40.47**	**184.34**	**0.945**	**0.179**	**0.306**	**2.02**	**0.838**	**1.63**	

Table J-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Clinical Chemistry
Male Rats

		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
ALB (g/dL)	Mean	3.42	3.33	3.43	3.46
	S.D.	0.215	0.128	0.149	0.227
	N	10	8	8	10
ALK P (U/L)	Mean	209.1	230.6	231.9	199.9
	S.D.	55.25	54.88	46.05	52.37
	N	10	8	8	10
ALT (U/L)	Mean	55.2	53.5	51.9	58.0
	S.D.	6.68	8.26	4.22	8.06
	N	10	8	8	10
AST (U/L)	Mean	88.3	80.6	82.9	104.5
	S.D.	13.14	14.97	18.14	52.01
	N	10	8	8	10
BUN (mg/dL)	Mean	14.1	12.9	12.9	13.5
	S.D.	3.52	2.03	2.10	1.90
	N	9	8	8	10
CA (mg/dL)	Mean	11.5	11.5	11.5	11.6
	S.D.	0.42	0.31	0.53	0.41
	N	10	8	8	10
CHOL (mg/dL)	Mean	62.5	71.8	70.1	64.8
	S.D.	14.03	15.31	11.26	13.41
	N	10	8	8	10
CREA (mg/dL)	Mean	0.52	0.51	0.56	0.46
	S.D.	0.079	0.113	0.092	0.070
	N	10	8	8	10
GLOB (g/dL)	Mean	3.41	3.24	3.36	3.44
	S.D.	0.242	0.213	0.873	0.158
	N	10	8	8	10
GLU (mg/dL)	Mean	205.1	210.6	209.5	216.3
	S.D.	58.98	53.68	61.34	41.46
	N	10	8	8	10
LDH (U/L)	Mean	604.3	576.0	517.8	755.6
	S.D.	342.39	203.23	109.40	419.99
	N	9	8	8	10
PHOS (mg/dL)	Mean	11.44	11.44	12.34	12.56
	S.D.	0.844	1.077	1.121	0.885
	N	10	8	8	10
TBIL (mg/dL)	Mean	0.74	0.65	0.54	0.90[a]
	S.D.	0.280	0.200	0.169	0.368
	N	10	8	8	10
TP (g/dL)	Mean	6.82	6.59	6.78	6.90
	S.D.	0.374	0.217	0.845	0.262
	N	10	8	8	10
Na (mmol/L)	Mean	151.9	150.6	151.8	152.6
	S.D.	1.81	1.60	1.33	1.52
	N	8	8	6	5
K (mmol/L)	Mean	10.01	9.69	9.68	9.58
	S.D.	0.923	1.348	0.454	0.864
	N	8	8	6	5
Cl (mmol/L)	Mean	104.3	102.6	103.7	104.0
	S.D.	1.28	1.06	1.51	1.22
	N	8	8	6	5

[a] = Significantly greater than 125 mg/kg-day group (p=0.023).

Table J-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Clinical Chemistry
Female Rats

		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
ALB (g/dL)	Mean	3.18	3.17	3.23	3.39
	S.D.	0.249	0.367	0.427	0.136
	N	8	9	10	8
ALK P (U/L)	Mean	155.5	152.2	135.0	128.4
	S.D.	79.93	62.20	32.62	64.17
	N	8	9	10	8
ALT (U/L)	Mean	88.8	106.0	88.0	89.3
	S.D.	15.51	49.40	24.29	23.15
	N	8	9	10	8
AST (U/L)	Mean	105.0	99.8	106.9	91.9
	S.D.	23.46	27.37	36.29	20.21
	N	8	9	10	8
BUN (mg/dL)	Mean	13.5	16.8	14.3	14.5
	S.D.	6.23	4.58	5.48	4.41
	N	8	9	10	8
CA (mg/dL)	Mean	11.9	12.0	11.8	12.2
	S.D.	0.65	0.58	0.51	0.25
	N	8	9	10	8
CHOL (mg/dL)	Mean	74.1	78.4	63.8	70.0
	S.D.	15.79	21.61	13.81	9.47
	N	8	9	10	8
CREA (mg/dL)	Mean	0.50	0.50	0.56	0.54
	S.D.	0.169	0.112	0.117	0.106
	N	8	9	10	8
GLOB (g/dL)	Mean	3.04	2.79	2.84	2.89
	S.D.	0.200	0.293	0.158	0.189
	N	8	9	10	8
GLU (mg/dL)	Mean	153.1	162.0	137.5	144.0
	S.D.	61.18	54.34	37.03	24.27
	N	8	9	10	8
LDH (U/L)	Mean	717.3	431.8	693.5	418.8
	S.D.	818.23	188.40	819.43	121.92
	N	8	9	10	8
PHOS (mg/dL)	Mean	11.65	12.00	11.25	13.38*
	S.D.	1.832	1.829	0.922	1.104
	N	8	9	10	8
TBIL (mg/dL)	Mean	0.46	0.40	0.26	0.26
	S.D.	0.515	0.283	0.207	0.151
	N	8	9	10	8
TP (g/dL)	Mean	6.21	5.94	6.08	6.26
	S.D.	0.449	0.550	0.489	0.267
	N	8	9	10	8
Na (mmol/L)	Mean	149.8	148.6	150.7	149.3
	S.D.	1.83	0.88	2.31	2.69
	N	8	9	10	7
K (mmol/L)	Mean	8.99	9.59	9.44	10.40
	S.D.	1.458	1.150	0.995	2.245
	N	8	9	10	7
Cl (mmol/L)	Mean	105.4	104.6	104.6	106.3
	S.D.	1.85	1.81	2.88	2.06
	N	8	9	10	7

* = Significantly greater than 125 mg/kg-day group (p=0.022).

Table J-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Clinical Chemistry
Recovery Male Rats

		Corn Oil Control	NTO in Corn Oil 500 mg/kg
ALB (g/dL)	Mean	3.53	3.46
	S.D.	0.125	0.178
	N	10	10
ALK P (U/L)	Mean	121.9	116.9
	S.D.	28.76	22.82
	N	10	10
ALT (U/L)	Mean	41.0	41.2
	S.D.	7.62	10.33
	N	10	10
AST (U/L)	Mean	71.8	64.9
	S.D.	14.73	17.33
	N	10	10
BUN (mg/dL)	Mean	17.0	18.0
	S.D.	2.45	6.36
	N	10	10
CA (mg/dL)	Mean	12.0	11.7
	S.D.	0.54	0.37
	N	10	10
CHOL (mg/dL)	Mean	96.1	92.2
	S.D.	21.54	16.75
	N	10	10
CREA (mg/dL)	Mean	0.61	0.56
	S.D.	0.110	0.097
	N	10	10
GLOB (g/dL)	Mean	3.24	3.32
	S.D.	0.207	0.230
	N	10	10
GLU (mg/dL)	Mean	253.1	222.8
	S.D.	43.04	40.47
	N	10	10
LDH (U/L)	Mean	405.5	373.4
	S.D.	98.38	184.34
	N	10	10
PHOS (mg/dL)	Mean	11.08	10.90
	S.D.	0.776	0.945
	N	10	10
TBIL (mg/dL)	Mean	0.47	0.39
	S.D.	0.170	0.179
	N	10	10
TP (g/dL)	Mean	6.78	6.75
	S.D.	0.230	0.306
	N	10	10
Na (mmol/L)	Mean	151.3	150.9
	S.D.	1.25	2.02
	N	10	10
K (mmol/L)	Mean	9.18	9.26
	S.D.	1.393	0.838
	N	10	10
Cl (mmol/L)	Mean	104.4	104.0
	S.D.	1.58	1.63
	N	10	10

Table J-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Clinical Chemistry Results
Non-Pregnant Female Rats

Dose	Animal ID	ALB (g/dL)	ALKP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREA (mg/dL)	GLOB (g/dL)	GLU (mg/dL)	LDH (U/L)	PHOS (mg/dL)	TBIL (mg/dL)	TP (g/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	NOTES
Control	12-0298[a]	3.5	182	73	79	13	12.3	80	0.5	2.7	197	335	9.2	0.3	6.3	149	8.7	103	
	12-0317[b]	4.1	124	29	92	15	11.3	62	0.5	3.0	126	550	11.2	0.7	7.1	153	10.3	106	
	Mean	**3.80**	**153.0**	**51.0**	**85.5**	**14.0**	**11.80**	**71.0**	**0.50**	**2.85**	**161.5**	**442.5**	**10.20**	**0.50**	**6.70**	**151.0**	**9.50**	**104.5**	
	SD	**0.424**	**41.01**	**31.11**	**9.19**	**1.41**	**0.707**	**12.73**	**0.000**	**0.212**	**50.20**	**152.03**	**1.414**	**0.283**	**0.566**	**2.83**	**1.131**	**2.12**	
31.25 mg/kg	12-0299[a]	3.8	77	47	78	16	11.6	62	0.8	3.2	155	374	8.9	< 0.1	7.0	154	8.5	105	
	Mean	**3.80**	**77.0**	**47.0**	**78.0**	**16.0**	**11.60**	**62.0**	**0.80**	**3.20**	**155.0**	**374.0**	**8.90**	**0.10**	**7.00**	**154.0**	**8.50**	**105.0**	
	SD																		
500 mg/kg	12-0171[a]	4.1	121	45	98	19	11.8	63	0.6	3.2	159	441	10.9	0.5	7.2	150	10.0	105	
	12-0229[a]	4.2	89	39	66	11	12.1	83	0.7	2.5	115	441	14.5	0.4	6.7	150	11.6	108	
	Mean	**4.15**	**105.0**	**42.0**	**82.0**	**15.0**	**11.95**	**73.0**	**0.65**	**2.85**	**137.0**	**441.0**	**12.70**	**0.45**	**6.95**	**150.0**	**10.80**	**106.5**	
	SD	**0.071**	**22.63**	**4.24**	**22.63**	**5.66**	**0.212**	**14.14**	**0.071**	**0.495**	**31.11**	**0.00**	**2.546**	**0.071**	**0.354**	**0.00**	**1.131**	**2.12**	

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.

Appendix K

Individual and Summary of Hematology Data

Table K-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Hematology Results
Male Rats

Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Control	12-0163	16.200	1.530	9.490	12.900	79.600	0.768	4.750	0.139	0.858	0.850	5.260	8.66	16.10	47.1	54.4	18.6	34.2	15.2	734.0	4.58
	12-0205	15.600	2.250	14.400	12.300	78.700	0.497	3.190	0.101	0.651	0.481	3.080	8.46	17.70	49.7	58.7	20.9	35.6	14.6	621.0	5.69
	12-0206	15.900	4.810	30.200	8.180	51.500	1.330	8.360	0.085	0.533	1.490	9.400	8.93	17.70	49.8	55.7	19.9	35.6	16.1	229.0	6.65
	12-0207	14.400	1.620	11.200	7.630	53.000	2.510	17.400	0.053	0.371	2.600	18.100	8.29	16.90	47.0	56.8	20.4	35.9	15.0	821.0	5.35
	12-0235	16.100	1.630	10.100	12.100	75.000	1.060	6.600	0.167	1.030	1.170	7.280	8.75	19.40	48.2	55.1	22.1	40.2	17.7	470.0	6.18
	12-0254	14.000	1.760	12.600	9.960	71.000	0.695	4.950	0.077	0.550	1.540	11.000	8.07	16.30	46.6	57.7	20.2	34.9	15.0	791.0	4.86
	12-0263	11.900	1.680	14.100	8.730	73.300	0.715	6.010	0.109	0.911	0.675	5.670	7.94	16.40	44.2	55.7	20.7	37.1	13.2	967.0	5.04
	12-0267	12.800	1.270	9.970	9.870	77.400	0.695	5.450	0.107	0.842	0.808	6.330	8.60	17.00	47.1	54.7	19.8	36.2	13.8	398.0	5.98
	12-0284	17.600	2.210	12.600	13.900	79.200	0.703	3.990	0.144	0.817	0.611	3.470	8.15	16.70	47.4	58.1	20.5	35.3	14.3	685.0	6.41
	12-0313	13.700	1.190	8.690	11.200	81.700	0.529	3.870	0.137	1.000	0.645	4.710	8.44	18.10	47.3	56.0	21.4	38.3	14.2	1093.0	5.46
	Mean	**14.8200**	**1.9950**	**13.3350**	**10.6770**	**72.0400**	**0.9502**	**6.4570**	**0.1119**	**0.7563**	**1.0870**	**7.4300**	**8.429**	**17.230**	**47.44**	**56.29**	**20.45**	**36.33**	**14.91**	**680.90**	**5.620**
	SD	**1.75613**	**1.04635**	**6.23552**	**2.12753**	**10.90700**	**0.60111**	**4.12636**	**0.03495**	**0.21898**	**0.64607**	**4.49093**	**0.3167**	**1.0100**	**1.595**	**1.477**	**0.949**	**1.775**	**1.266**	**262.166**	**0.6853**
31.25 mg/kg	12-0164	9.200	1.180	12.800	6.940	75.400	0.377	4.090	0.082	0.893	0.620	6.730	8.96	16.50	47.6	53.1	18.4	34.6	14.7	570.0	5.10
	12-0165	14.700	1.150	7.810	11.900	81.200	0.540	3.680	0.051	0.350	1.020	6.960	8.18	15.80	45.0	55.0	19.3	35.2	14.9	409.0	6.22
	12-0225	7.040	1.070	15.200	5.310	75.500	0.321	4.560	0.059	0.832	0.277	3.940	8.10	16.70	44.1	54.4	20.5	37.8	14.0	659.0	5.67
	12-0234	18.400	1.980	10.700	13.500	73.300	1.270	6.930	0.125	0.682	1.530	8.310	9.08	18.00	50.0	55.1	19.9	36.1	16.8	868.0	5.10
	12-0243	18.000	1.260	7.000	14.600	80.700	0.659	3.660	0.166	0.918	1.390	7.700	8.44	16.80	47.0	55.7	19.9	35.7	14.5	913.0	4.95
	12-0245	12.300	1.570	12.800	9.060	73.900	0.816	6.650	0.139	1.130	0.684	5.580	8.13	16.10	44.7	55.0	19.8	36.1	14.9	574.0	6.23
	12-0255	15.300	1.430	9.330	11.500	75.600	1.210	7.940	0.089	0.584	1.010	6.580	8.73	17.20	47.0	53.9	19.7	36.6	16.4	819.0	5.45
	12-0262	13.000	1.980	15.300	8.940	69.000	0.719	5.540	0.119	0.918	1.200	9.280	7.89	16.50	44.0	55.8	20.9	37.5	15.3	1076.0	4.94
	12-0324	19.400	1.210	6.230	16.900	87.100	0.582	3.000	0.155	0.801	0.558	2.880	8.46	16.20	44.9	53.1	19.1	36.0	15.9	450.0	5.57
	12-0351	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)	(f)
	Mean	**14.1489**	**1.4256**	**10.7967**	**10.9611**	**76.8556**	**0.7216**	**5.1167**	**0.1094**	**0.7898**	**0.9210**	**6.4400**	**8.441**	**16.644**	**46.03**	**54.57**	**19.72**	**36.18**	**15.27**	**704.22**	**5.470**
	SD	**4.21118**	**0.34861**	**3.42866**	**3.74054**	**5.32943**	**0.33238**	**1.72554**	**0.04125**	**0.22599**	**0.41528**	**2.03677**	**0.4102**	**0.6540**	**2.002**	**1.015**	**0.740**	**1.017**	**0.923**	**226.552**	**0.5019**
125 mg/kg	12-0187	6.970	0.972	14.000	5.460	78.300	0.313	4.500	0.026	0.372	0.198	2.850	8.48	16.40	46.2	54.5	19.3	35.4	16.0	1023.0	5.19
	12-0204	12.700	1.280	10.100	9.970	78.700	0.367	2.900	0.081	0.636	0.975	7.700	8.10	16.50	46.7	57.6	20.3	35.3	15.3	959.0	5.83
	12-0223	12.900	2.190	17.000	8.140	63.200	0.855	6.640	0.169	1.310	1.530	11.900	9.08	17.80	47.9	52.7	19.6	37.2	16.2	663.0	5.16
	12-0224	12.900	1.490	11.500	9.660	74.900	0.807	6.260	0.169	1.310	0.774	6.000	7.96	15.30	42.6	53.5	19.3	36.0	15.7	1002.0	5.22
	12-0253	15.600	3.550	22.800	9.860	63.300	1.130	7.230	0.048	0.310	0.995	6.390	8.66	17.70	48.8	56.3	20.5	36.3	14.8	713.0	5.07
	12-0275	10.900	1.930	17.700	7.420	68.200	0.844	7.750	0.120	1.110	0.573	5.270	8.98	17.60	48.6	54.2	19.6	36.3	14.6	968.0	4.95
	12-0293	27.600	2.730	9.870	22.000	79.700	1.260	4.540	0.237	0.859	1.380	4.980	8.29	18.20	47.1	56.8	22.0	38.7	16.0	692.0	5.64
	12-0296	18.700	2.380	12.700	14.400	76.900	0.927	4.950	0.175	0.935	0.837	4.470	8.59	18.40	48.4	56.4	21.5	38.0	15.2	916.0	5.39
	12-0345	17.900	2.090	11.700	12.500	69.700	1.630	9.110	0.149	0.829	1.550	8.660	8.41	17.50	45.8	54.4	20.8	38.2	16.3	1368.0	5.71
	12-0354	20.100	2.600	12.900	16.100	80.100	0.556	2.770	0.127	0.630	0.716	3.560	8.74	17.80	49.5	56.6	20.4	35.9	15.3	482.0	5.64
	Mean	**15.6270**	**2.1212**	**14.0270**	**11.5510**	**73.3000**	**0.8689**	**5.6650**	**0.1301**	**0.8301**	**0.9528**	**6.1780**	**8.529**	**17.320**	**47.16**	**55.30**	**20.33**	**36.73**	**15.54**	**878.60**	**5.380**
	SD	**5.76098**	**0.75846**	**4.03911**	**4.87200**	**6.64881**	**0.40377**	**2.08512**	**0.06405**	**0.35101**	**0.43333**	**2.67501**	**0.3581**	**0.9578**	**2.003**	**1.636**	**0.919**	**1.215**	**0.589**	**248.878**	**0.3051**
500 mg/kg	12-0177	18.700	1.270	6.800	15.700	83.900	0.785	4.190	0.128	0.681	0.837	4.470	7.81	16.20	44.6	57.1	20.8	36.4	15.3	626.0	6.22
	12-0189	13.200	1.780	13.500	9.660	72.900	0.571	4.310	0.224	1.690	1.010	7.650	9.04	17.10	48.2	53.4	18.9	35.5	15.1	594.0	5.74
	12-0203	11.600	0.989	8.520	9.330	80.300	0.477	4.110	0.073	0.631	0.751	6.460	8.51	17.70	49.4	58.0	20.8	35.8	15.5	676.0	5.82
	12-0209	2.540	0.497	19.600	1.750	68.900	0.128	5.040	0.007	0.290	0.157	6.200	7.64	16.30	46.1	60.3	21.3	35.3	14.9	870.0	5.49
	12-0236	15.200	1.510	9.920	10.300	67.900	1.330	8.710	0.191	1.260	1.860	12.200	8.39	18.20	47.3	56.3	21.7	38.5	16.8	372.0	6.52
	12-0256	12.100	1.330	11.000	9.270	76.600	0.808	6.670	0.086	0.715	0.609	5.030	8.95	19.00	49.5	55.3	21.2	38.4	15.8	464.0	5.35
	12-0297	16.900	2.580	15.200	10.300	61.000	1.650	9.750	0.283	1.680	2.090	12.300	7.84	17.00	45.2	57.6	21.7	37.6	14.2	192.0	5.37
	12-0314	19.700	2.180	11.100	15.500	78.900	0.819	4.160	0.262	1.330	0.883	4.490	8.39	18.90	47.9	57.1	22.5	39.5	16.1	528.0	5.81
	12-0322	21.300	2.110	9.900	16.400	77.300	1.300	6.130	0.213	1.000	1.220	5.710	8.69	18.00	47.6	54.9	20.7	37.7	14.5	455.0	5.56
	12-0341	12.000	1.970	16.400	7.810	65.100	1.090	9.050	0.080	0.662	1.060	8.800	8.21	17.50	45.6	55.6	21.4	38.5	15.6	899.0	4.90
	Mean	**14.3240**	**1.6216**	**12.1940**	**10.6020**	**73.2800**	**0.8958**	**6.2120**	**0.1547**	**0.9939**	**1.0477**	**7.3310**	**8.347**	**17.590**	**47.14**	**56.56**	**21.10**	**37.32**	**15.38**	**567.60**	**5.678**
	SD	**5.39559**	**0.62373**	**3.92896**	**4.39965**	**7.35464**	**0.45396**	**2.22952**	**0.09249**	**0.47751**	**0.56988**	**2.92260**	**0.4779**	**0.9689**	**1.709**	**1.916**	**0.943**	**1.471**	**0.764**	**216.396**	**0.4596**

(f) = Animal died on study

Table K-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Hematology Results
Female Rats

Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Control	12-0211	5.360	1.340	25.100	3.780	70.500	0.166	3.100	0.007	0.125	0.062	1.170	7.20	14.90	42.4	58.9	20.7	35.1	16.0	857.0	5.27
	12-0220	15.500	6.540	42.100	6.860	44.200	1.250	8.050	0.115	0.741	0.758	4.880	6.34	13.10	35.7	56.4	20.6	36.6	15.7	658.0	5.44
	12-0222	15.100	2.830	18.800	11.400	75.900	0.429	2.850	0.069	0.460	0.310	2.060	6.97	14.60	39.7	56.9	21.0	36.8	16.8	562.0	5.29
	12-0260	13.500	3.620	26.900	8.050	59.700	0.727	5.390	0.041	0.301	1.050	7.750	7.48	15.10	42.1	56.3	20.2	35.9	17.4	957.0	4.78
	12-0289	19.700	5.240	26.600	13.000	66.200	1.050	5.320	0.108	0.547	0.247	1.250	6.58	14.30	37.1	56.4	21.7	38.4	15.3	1206.0	5.33
	12-0298[a,d]																				
	12-0306	15.300	4.780	31.200	8.490	55.400	1.220	7.950	0.035	0.226	0.807	5.260	6.97	13.30	35.5	50.9	19.1	37.6	14.8	1355.0	5.44
	12-0309	12.200	5.550	45.500	4.910	40.200	1.020	8.380	0.051	0.421	0.673	5.510	6.28	12.90	33.7	53.6	20.5	38.2	15.2	1133.0	5.35
	12-0317[b]																				
	12-0339	5.730	1.130	19.800	2.880	50.300	1.410	24.600	0.032	0.551	0.271	4.730	6.76	13.70	37.6	55.5	20.2	36.4	16.6	1141.0	5.00
	Mean	**12.7988**	**3.8788**	**29.5000**	**7.4213**	**57.8000**	**0.9090**	**8.2050**	**0.0573**	**0.4215**	**0.5223**	**4.0763**	**6.823**	**13.988**	**37.98**	**55.61**	**20.50**	**36.88**	**15.98**	**983.63**	**5.238**
	SD	**4.96601**	**1.98955**	**9.71567**	**3.56558**	**12.64798**	**0.43289**	**6.96830**	**0.03781**	**0.19883**	**0.34510**	**2.34600**	**0.4154**	**0.8509**	**3.164**	**2.404**	**0.745**	**1.135**	**0.896**	**276.455**	**0.2308**
31.25 mg/kg	12-0168	9.320	2.250	24.200	5.990	64.300	0.672	7.200	0.037	0.395	0.366	3.920	7.11	14.10	39.2	55.0	19.8	36.0	15.9	888.0	4.98
	12-0170	7.480	3.180	42.500	2.950	39.400	0.757	10.100	0.049	0.656	0.550	7.350	6.58	13.10	37.5	57.0	19.9	34.9	16.7	953.0	4.84
	12-0201[c]	7.110	2.730	38.300	3.490	49.100	0.562	7.900	0.022	0.303	0.310	4.360	7.33	14.50	39.2	53.4	19.7	36.9	15.9	1073.0	6.21
	12-0221	10.700	1.900	17.700	8.160	76.000	0.415	3.870	0.040	3.720	0.215	2.000	5.92	11.70	31.7	53.6	19.7	36.8	14.9	1285.0	5.19
	12-0240	15.100	4.030	26.700	10.500	69.600	0.386	2.560	0.041	0.274	0.136	0.898	7.28	14.70	40.2	55.2	20.2	36.5	17.4	1300.0	5.13
	12-0299[a]																				
	12-0300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	12-0346	11.500	4.270	37.000	5.010	43.400	1.340	11.600	0.037	0.321	0.882	7.650	8.17	16.60	43.9	53.7	20.4	37.9	16.9	666.0	5.24
	12-0367	16.500	2.700	16.300	12.900	78.000	0.601	3.640	0.072	0.437	0.273	1.650	7.20	14.80	39.2	54.5	20.6	37.7	14.7	697.0	4.90
	12-0369	7.910	1.490	18.900	5.230	66.100	0.859	10.900	0.047	0.590	0.282	3.570	6.96	13.70	38.2	54.8	19.7	35.8	14.8	1130.0	5.45
	Mean	**10.7025**	**2.8188**	**27.7000**	**6.7788**	**60.7375**	**0.6990**	**7.2213**	**0.0431**	**0.8370**	**0.3768**	**3.9248**	**7.069**	**14.150**	**38.64**	**54.65**	**20.00**	**36.56**	**15.90**	**999.00**	**5.243**
	SD	**3.51700**	**0.97533**	**10.27160**	**3.48158**	**14.84317**	**0.30416**	**3.53025**	**0.01424**	**1.17286**	**0.23716**	**2.50615**	**0.6453**	**1.4223**	**3.395**	**1.169**	**0.355**	**0.994**	**1.038**	**242.477**	**0.4380**
125 mg/kg	12-0169	5.930	0.735	12.400	4.920	83.000	0.191	3.230	0.060	1.020	0.022	0.378	7.14	14.50	42.3	59.2	20.2	34.2	19.9	1041.0	4.71
	12-0210	6.050	1.010	16.700	4.550	75.200	0.301	4.970	0.020	0.334	0.170	2.800	7.62	16.00	44.9	58.9	21.0	35.6	17.6	1220.0	5.54
	12-0212	7.250	1.060	14.700	5.550	76.500	0.502	6.930	0.018	0.246	0.119	1.650	7.01	14.70	41.0	58.4	20.9	35.8	16.1	776.0	5.08
	12-0227	5.490	1.870	34.000	3.130	57.000	0.355	6.470	0.017	0.304	0.122	2.230	6.62	13.10	33.8	51.0	19.8	38.8	17.1	1205.0	5.24
	12-0257	7.170	1.780	24.900	4.440	62.000	0.819	11.400	0.035	0.490	0.091	1.260	6.70	14.20	38.8	58.0	21.2	36.6	18.8	939.0	5.19
	12-0259	11.100	3.410	30.700	6.130	55.100	1.190	10.700	0.017	0.156	0.370	3.320	7.91	15.90	45.0	56.9	20.1	35.3	17.4	1085.0	4.26
	12-0261	15.100	3.570	23.600	9.840	65.200	1.160	7.670	0.052	0.343	0.477	3.160	7.05	14.60	40.0	56.7	20.8	36.6	17.3	1043.0	5.37
	12-0278	9.270	2.430	26.200	5.430	58.500	0.952	10.300	0.040	0.433	0.428	4.620	7.21	14.30	39.1	54.3	19.8	36.5	16.8	1565.0	5.07
	12-0291	20.900	10.300	49.400	8.340	39.900	1.270	6.090	0.073	0.351	0.882	4.220	5.84	12.10	32.8	56.2	20.8	36.9	14.8	1465.0	4.91
	12-0327	10.100	3.700	36.500	5.430	53.500	0.708	6.980	0.040	0.395	0.261	2.570	6.91	13.50	38.9	56.3	19.6	34.7	14.9	1215.0	5.38
	Mean	**9.8360**	**2.9865**	**26.9100**	**5.7760**	**62.5900**	**0.7448**	**7.4740**	**0.0372**	**0.4072**	**0.2942**	**2.6208**	**7.001**	**14.290**	**39.66**	**56.59**	**20.42**	**36.10**	**17.07**	**1155.40**	**5.075**
	SD	**4.88061**	**2.79315**	**11.26336**	**1.95869**	**12.81392**	**0.39592**	**2.61598**	**0.01978**	**0.23466**	**0.25708**	**1.30578**	**0.6644**	**1.1865**	**4.054**	**2.455**	**0.583**	**1.297**	**1.575**	**234.582**	**0.3744**
500 mg/kg	12-0166	10.100	4.430	43.700	4.070	40.200	1.190	11.700	0.043	0.422	0.399	3.940	6.92	14.20	40.0	57.7	20.5	35.5	17.4	1253.0	4.91
	12-0167	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	12-0171[a]																				
	12-0198	8.340	4.140	49.600	2.950	35.300	0.771	9.250	0.022	0.259	0.465	5.570	7.33	14.70	40.8	55.7	20.1	36.1	16.1	586.0	6.14
	12-0229[a]																				
	12-0258	6.360	1.450	22.800	3.600	56.600	0.701	11.000	0.045	0.710	0.566	8.890	7.23	15.10	40.1	55.5	20.8	37.5	18.3	1162.0	5.19
	12-0279	13.200	6.650	50.300	4.970	37.600	0.979	7.410	0.080	0.601	0.549	4.150	6.39	13.40	34.6	54.2	20.9	38.6	15.9	516.0	4.66
	12-0308[c]	14.500	2.950	20.300	7.990	55.100	2.950	20.300	0.055	0.378	0.569	3.920	7.14	15.10	39.3	55.1	21.2	38.4	15.9	1108.0	4.98
	12-0336	9.420	2.530	26.900	5.400	57.300	0.541	5.740	0.106	1.120	0.838	8.900	7.28	13.70	40.7	55.9	18.8	33.6	16.7	1257.0	6.07
	12-0348	9.970	3.510	35.200	4.430	44.500	1.360	13.600	0.420	0.416	0.625	6.270	6.29	15.10	36.3	57.7	24.0	41.6	16.3	929.0	5.24
	Mean	**10.2700**	**3.6657**	**35.5429**	**4.7729**	**46.6571**	**1.2131**	**11.2857**	**0.1101**	**0.5580**	**0.5730**	**5.9486**	**6.940**	**14.471**	**38.83**	**55.97**	**20.90**	**37.33**	**16.66**	**973.00**	**5.313**
	SD	**2.77885**	**1.65584**	**12.59270**	**1.63769**	**9.49366**	**0.81682**	**4.77470**	**0.13934**	**0.28904**	**0.13875**	**2.19825**	**0.4315**	**0.7135**	**2.411**	**1.302**	**1.577**	**2.575**	**0.898**	**309.181**	**0.5740**

ND = No data
a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but dam was pregnant.
d= Animal was not fasted prior to necropsy.

Table K-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Hematology Results
Recovery Male Rats

Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Control	12-0174	11.800	0.942	7.970	9.240	78.200	0.864	7.310	0.130	1.100	0.637	5.390	8.18	15.60	42.3	51.7	19.0	36.8	16.2	1009.0	4.71
	12-0179	20.200	1.630	8.090	16.800	83.300	0.922	4.570	0.148	0.733	0.667	3.310	7.52	14.80	41.0	54.5	19.7	36.2	15.2	1244.0	4.79
	12-0194	12.500	1.200	9.660	9.210	74.000	0.971	7.800	0.176	1.410	0.888	7.130	8.88	16.30	45.1	50.9	18.4	36.1	16.6	823.0	5.34
	12-0208	11.800	0.852	7.230	9.870	83.700	0.604	5.120	0.094	0.799	0.368	3.120	8.36	16.20	44.8	53.6	19.3	36.1	16.6	747.0	4.91
	12-0272	13.900	0.866	6.250	11.100	80.400	1.010	7.300	0.171	1.240	0.671	4.840	7.92	15.20	42.3	53.4	19.2	36.0	15.8	958.0	5.77
	12-0294	19.600	1.970	10.000	14.500	74.200	1.700	8.700	0.327	1.670	1.050	5.350	8.72	15.90	44.3	50.9	18.3	36.0	17.2	961.0	5.66
	12-0304	20.300	1.860	9.150	16.000	78.500	1.490	7.310	0.292	1.440	0.738	3.630	9.08	16.30	45.1	49.7	18.0	36.2	17.2	883.0	5.79
	12-0323	11.300	0.905	8.020	8.830	78.300	0.878	7.780	0.169	1.500	0.499	4.420	7.44	13.90	39.0	52.4	18.7	35.6	15.0	1433.0	5.62
	12-0332	8.320	1.300	15.600	5.430	65.200	0.906	10.900	0.129	1.550	0.561	6.740	8.92	16.60	46.9	52.5	18.6	35.4	15.4	877.0	5.25
	12-0365	14.800	1.730	11.700	10.900	73.500	1.080	7.290	0.304	2.050	0.803	5.430	8.82	16.90	46.4	52.5	19.2	36.5	17.7	922.0	4.82
	Mean	**14.4520**	**1.3255**	**9.3670**	**11.1880**	**76.9300**	**1.0425**	**7.4080**	**0.1940**	**1.3492**	**0.6882**	**4.9360**	**8.384**	**15.770**	**43.72**	**52.21**	**18.84**	**36.09**	**16.29**	**985.70**	**5.266**
	SD	**4.21082**	**0.43882**	**2.67719**	**3.55698**	**5.47521**	**0.32050**	**1.74768**	**0.08253**	**0.39798**	**0.19560**	**1.35861**	**0.5978**	**0.9141**	**2.501**	**1.445**	**0.527**	**0.398**	**0.927**	**205.098**	**0.4316**
500 mg/kg	12-0176	13.000	1.830	14.000	9.160	70.500	0.876	6.740	0.261	2.010	0.869	6.690	8.90	15.70	43.9	49.3	17.7	35.9	17.2	992.0	5.04
	12-0186	12.200	0.802	6.580	10.300	84.600	0.468	3.840	0.074	0.606	0.533	4.380	8.64	15.40	43.0	49.8	17.9	35.9	17.5	1020.0	4.94
	12-0215	19.700	1.730	8.800	16.200	82.600	0.656	3.340	0.269	1.370	0.764	3.890	7.65	14.80	40.2	52.5	19.3	36.7	17.4	1255.0	5.62
	12-0244	16.500	1.210	7.320	13.900	84.300	0.802	4.860	0.127	0.773	0.444	2.690	8.60	15.90	44.8	52.1	18.5	35.6	15.6	889.0	4.96
	12-0283	5.620	0.979	17.400	4.080	72.600	0.365	6.500	0.052	0.923	0.143	2.550	7.96	15.90	44.1	55.4	20.0	36.1	16.6	967.0	5.13
	12-0295	19.600	1.740	8.860	15.300	78.000	1.570	8.020	0.222	1.130	0.778	3.970	7.74	15.20	42.1	54.4	19.7	36.1	15.2	1135.0	5.09
	12-0302	11.800	1.040	8.800	9.150	77.300	1.020	8.580	0.182	1.530	0.453	3.820	9.23	17.00	47.1	51.0	18.4	36.0	16.9	906.0	4.79
	12-0310	13.000	1.690	13.000	9.090	70.100	1.290	9.930	0.254	1.960	0.643	4.960	7.69	15.20	42.8	55.7	19.8	35.6	15.3	982.0	5.49
	12-0333	17.100	1.600	9.390	14.000	81.800	0.808	4.740	0.161	0.944	0.542	3.170	8.34	16.10	45.1	54.0	19.3	35.8	16.0	947.0	5.13
	12-0363	13.300	1.350	10.100	10.100	76.000	0.971	7.290	0.228	1.710	0.651	4.890	8.03	15.70	43.1	53.7	19.5	36.3	15.6	1022.0	4.77
	Mean	**14.1820**	**1.3971**	**10.4250**	**11.1280**	**77.7800**	**0.8826**	**6.3840**	**0.1830**	**1.2956**	**0.5820**	**4.1010**	**8.278**	**15.690**	**43.62**	**52.79**	**19.01**	**36.00**	**16.33**	**1011.50**	**5.096**
	SD	**4.21865**	**0.37065**	**3.35649**	**3.68810**	**5.48408**	**0.36032**	**2.15503**	**0.07798**	**0.49694**	**0.20944**	**1.22679**	**0.5501**	**0.6082**	**1.866**	**2.237**	**0.821**	**0.330**	**0.893**	**109.674**	**0.2745**

(f) = Animal died on study

Table K-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Hematology
Male Rats

		Corn Oil	NTO in Corn Oil		
		Control	31.25 mg/kg	125 mg/kg	500 mg/kg
WBC	Mean	14.8200	14.1489	15.6270	14.3240
(K/uL)	S.D.	1.75613	4.21118	5.76098	5.39559
	N	10	9	10	10
NEU	Mean	13.3350	10.7967	14.0270	12.1940
(%N)	S.D.	6.23552	3.42866	4.03911	3.92896
	N	10	9	10	10
LYM	Mean	72.0400	76.8556	73.3000	73.2800
(%L)	S.D.	10.90700	5.32943	6.64881	7.35464
	N	10	9	10	10
MONO	Mean	6.4570	5.1167	5.6650	6.2120
(%M)	S.D.	4.12636	1.72554	2.08512	2.22952
	N	10	9	10	10
EOS	Mean	0.7563	0.7898	0.8301	0.9939
(%E)	S.D.	0.21898	0.22599	0.35101	0.47751
	N	10	9	10	10
BASO	Mean	7.4300	6.4400	6.1780	7.3310
(%B)	S.D.	4.49093	2.03677	2.67501	2.92260
	N	10	9	10	10
RBC	Mean	8.429	8.441	8.529	8.347
(M/uL)	S.D.	0.3167	0.4102	0.3581	0.4779
	N	10	9	10	10
HGB	Mean	17.230	16.644	17.320	17.590
(g/dL)	S.D.	1.0100	0.6540	0.9578	0.9689
	N	10	9	10	10
HCT	Mean	47.44	46.03	47.16	47.14
(%)	S.D.	1.595	2.002	2.003	1.709
	N	10	9	10	10
MCV	Mean	56.29	54.57	55.30	56.56[a]
(fL)	S.D.	1.477	1.015	1.636	1.916
	N	10	9	10	10
MCH	Mean	20.45	19.72	20.33	21.10[b]
(pg)	S.D.	0.949	0.740	0.919	0.943
	N	10	9	10	10
MCHC	Mean	36.33	36.18	36.73	37.32
(g/dL)	S.D.	1.775	1.017	1.215	1.471
	N	10	9	10	10
RDW	Mean	14.91	15.27	15.54	15.38
(%)	S.D.	1.266	0.923	0.589	0.764
	N	10	9	10	10
PLT	Mean	680.90	704.22	878.60	567.60[c]
(K/uL)	S.D.	262.166	226.552	248.878	216.396
	N	10	9	10	10
MPV	Mean	5.620	5.470	5.380	5.678
(fL)	S.D.	0.6853	0.5019	0.3051	0.4596
	N	10	9	10	10

[a] = Significantly greater than 31.25 mg/kg-day group (p=0.041).
[b] = Significantly greater than 31.25 mg/kg-day group (p=0.010).
[c] = Signficantly less than 125 mg/kg-day group (p=0.031).

Table K-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Hematology
Female Rats

		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	125 mg/kg	500 mg/kg
WBC	Mean	12.7988	10.7025	9.8360	10.2700
(K/uL)	S.D.	4.96601	3.51700	4.88061	2.77885
	N	8	8	10	7
NEU	Mean	29.5000	27.7000	26.9100	35.5429
(%N)	S.D.	9.71567	10.27160	11.26336	12.59270
	N	8	8	10	7
LYM	Mean	57.8000	60.7375	62.5900	46.6571
(%L)	S.D.	12.64798	14.84317	12.81392	9.49366
	N	8	8	10	7
MONO	Mean	8.2050	7.2213	7.4740	11.2857
(%M)	S.D.	6.96830	3.53025	2.61598	4.77470
	N	8	8	10	7
EOS	Mean	0.4215	0.8370	0.4072	0.5580
(%E)	S.D.	0.19883	1.17286	0.23466	0.28904
	N	8	8	10	7
BASO	Mean	4.0763	3.9248	2.6208	5.9486[a]
(%B)	S.D.	2.34600	2.50615	1.30578	2.19825
	N	8	8	10	7
RBC	Mean	6.823	7.069	7.001	6.940
(M/uL)	S.D.	0.4154	0.6453	0.5644	0.4315
	N	8	8	10	7
HGB	Mean	13.988	14.150	14.290	14.471
(g/dL)	S.D.	0.8509	1.4223	1.1865	0.7135
	N	8	8	10	7
HCT	Mean	37.98	38.64	39.66	38.83
(%)	S.D.	3.164	3.395	4.054	2.411
	N	8	8	10	7
MCV	Mean	55.61	54.65	56.59	55.97
(fL)	S.D.	2.404	1.169	2.455	1.302
	N	8	8	10	7
MCH	Mean	20.50	20.00	20.42	20.90
(pg)	S.D.	0.745	0.355	0.583	1.577
	N	8	8	10	7
MCHC	Mean	36.88	36.56	36.10	37.33
(g/dL)	S.D.	1.135	0.994	1.297	2.575
	N	8	8	10	7
RDW	Mean	15.98	15.90	17.07	16.66
(%)	S.D.	0.896	1.038	1.575	0.898
	N	8	8	10	7
PLT	Mean	983.63	999.00	1155.40	973.00
(K/uL)	S.D.	276.455	242.477	234.582	309.181
	N	8	8	10	7
MPV	Mean	5.238	5.243	5.075	5.313
(fL)	S.D.	0.2308	0.4380	0.3744	0.5740
	N	8	8	10	7

[a] = Significantly greater than the 125 mg/kg-day group (p=0.015).

Table K-6
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Hematology
Recovery Male Rats

		Corn Oil Control	NTO in Corn Oil 500 mg/kg
WBC (K/uL)	Mean	14.4520	14.1820
	S.D.	4.21082	4.21865
	N	10	10
NEU (%N)	Mean	9.3670	10.4250
	S.D.	2.67719	3.35649
	N	10	10
LYM (%L)	Mean	76.9300	77.7800
	S.D.	5.47521	5.48408
	N	10	10
MONO (%M)	Mean	7.4080	6.3840
	S.D.	1.74768	2.15503
	N	10	10
EOS (%E)	Mean	1.3492	1.2956
	S.D.	0.39798	0.49694
	N	10	10
BASO (%B)	Mean	4.9360	4.1010
	S.D.	1.35861	1.22679
	N	10	10
RBC (M/uL)	Mean	8.384	8.278
	S.D.	0.5978	0.5501
	N	10	10
HGB (g/dL)	Mean	15.770	15.690
	S.D.	0.9141	0.6082
	N	10	10
HCT (%)	Mean	43.72	43.62
	S.D.	2.501	1.866
	N	10	10
MCV (fL)	Mean	52.21	52.79
	S.D.	1.445	2.237
	N	10	10
MCH (pg)	Mean	18.84	19.01
	S.D.	0.527	0.821
	N	10	10
MCHC (g/dL)	Mean	36.09	36.00
	S.D.	0.398	0.330
	N	10	10
RDW (%)	Mean	16.29	16.33
	S.D.	0.927	0.893
	N	10	10
PLT (K/uL)	Mean	985.70	1011.50
	S.D.	205.098	109.674
	N	10	10
MPV (fL)	Mean	5.266	5.096
	S.D.	0.4316	0.2745
	N	10	10

Table K-7
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Hematology Results
Non-Pregnant Female Rats

Group	Animal ID	WBC (K/uL)	NEU (K/uL)	NEU (%N)	LYM (K/uL)	LYM (%L)	MONO (K/uL)	MONO (%M)	EOS (K/uL)	EOS (%E)	BASO (K/uL)	BASO (%B)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/uL)	MPV (fL)
Corn Oil Control	12-0298[a,d]	12.900	2.580	20.100	8.920	69.400	0.596	4.640	0.161	1.250	0.597	4.650	7.27	15.50	41.1	56.5	21.3	37.8	14.2	1087.0	5.68
	12-0317[b]	8.870	0.499	5.620	7.680	86.600	0.328	3.700	0.098	1.100	0.265	2.980	8.54	17.50	46.7	54.7	20.5	37.4	14.4	1026.0	5.57
	Mean	**10.8850**	**1.5395**	**12.8600**	**8.3000**	**78.0000**	**0.4620**	**4.1700**	**0.1295**	**1.1750**	**0.4310**	**3.8150**	**7.905**	**16.500**	**43.90**	**55.60**	**20.90**	**37.60**	**14.30**	**1056.50**	**5.625**
	SD	**2.84964**	**1.47149**	**10.23891**	**0.87681**	**12.16224**	**0.18950**	**0.66468**	**0.04455**	**0.10607**	**0.23476**	**1.18087**	**0.8980**	**1.4142**	**3.960**	**1.273**	**0.566**	**0.283**	**0.141**	**43.134**	**0.0778**
31.25 mg/kg	12-0299[a]	11.600	0.910	7.830	10.300	88.500	0.156	1.340	0.079	0.679	0.190	1.630	7.79	16.10	43.4	55.8	20.7	37.1	14.8	1176.0	5.62
	Mean	**11.6000**	**0.9100**	**7.8300**	**10.3000**	**88.5000**	**0.1560**	**1.3400**	**0.0790**	**0.6790**	**0.1900**	**1.6300**	**7.790**	**16.100**	**43.40**	**55.80**	**20.70**	**37.10**	**14.80**	**1176.00**	**5.620**
	SD																				
500 mg/kg	12-0171[a]	11.700	0.532	4.560	10.100	86.300	0.475	4.070	0.099	0.844	0.492	4.210	8.54	16.20	46.0	53.9	19.0	35.2	16.5	1180.0	5.13
	12-0229[a]	4.620	0.293	6.340	3.900	84.500	0.197	4.280	0.420	0.899	0.185	4.010	8.41	15.50	43.4	51.6	18.4	35.6	15.9	1027.0	5.05
	Mean	**8.1600**	**0.4125**	**5.4500**	**7.0000**	**85.4000**	**0.3360**	**4.1750**	**0.2595**	**0.8715**	**0.3385**	**4.1100**	**8.475**	**15.850**	**44.70**	**52.75**	**18.70**	**35.40**	**16.20**	**1103.50**	**5.090**
	SD	**5.00632**	**0.16900**	**1.25865**	**4.38406**	**1.27279**	**0.19658**	**0.14849**	**0.22698**	**0.03889**	**0.21708**	**0.14142**	**0.0919**	**0.4950**	**1.838**	**1.626**	**0.424**	**0.283**	**0.424**	**108.187**	**0.0566**

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
d= Animal was not fasted prior to necropsy.

Appendix L

Individual and Summary of Prothrombin Time Data

Table L-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Prothrombin Time Results
Male Rats

Dose	Animal ID	PT Prothrombin Time (sec) Channel A	Channel B	Average	NOTES
Control	12-0163	10.5	11.4	**11.0**	plasma slightly hemolyzed/lipemic
	12-0205	10.5	10.1	**10.3**	plasma slightly hemolyzed/lipemic
	12-0206	9.5	9.6	**9.6**	plasma slightly hemolyzed/lipemic
	12-0207	9.5	9.7	**9.6**	plasma slightly hemolyzed/lipemic
	12-0235	9.8	9.5	**9.7**	plasma hemolyzed/lipemic
	12-0254	9.9	9.8	**9.9**	plasma slightly hemolyzed/lipemic
	12-0263	10.0	9.7	**9.9**	plasma slightly hemolyzed/lipemic
	12-0267	9.7	9.3	**9.5**	plasma slightly hemolyzed/lipemic
	12-0284	10.2	11.0	**10.6**	plasma slightly hemolyzed/lipemic
	12-0313	7.5	9.4	**8.5**	plasma slightly hemolyzed/lipemic
	Mean	**9.71**	**9.95**	**9.83**	
	SD	**0.856**	**0.701**	**0.687**	
31.25 mg/kg	12-0164	10.7	10.4	**10.6**	plasma slightly hemolyzed/lipemic
	12-0165	10.3	10.4	**10.4**	plasma hemolyzed/lipemic
	12-0225	10.0	9.5	**9.8**	plasma hemolyzed/lipemic
	12-0234	10.2	10.0	**10.1**	plasma slightly lipemic
	12-0243	9.8	9.6	**9.7**	plasma normal
	12-0245	8.4	9.7	**9.1**	plasma slightly hemolyzed/lipemic
	12-0255	8.5	8.4	**8.5**	plasma slightly hemolyzed/lipemic
	12-0262	9.7	10.2	**10.0**	plasma slightly hemolyzed/lipemic
	12-0324	9.6	9.2	**9.4**	plasma slightly lipemic
	12-0351	(f)	(f)	**(f)**	
	Mean	**9.69**	**9.71**	**9.70**	
	SD	**0.779**	**0.643**	**0.656**	
125 mg/kg	12-0187	10.0	10.1	**10.1**	plasma slightly hemolyzed/lipemic
	12-0204	9.8	9.7	**9.8**	plasma normal
	12-0223	9.4	8.6	**9.0**	plasma hemolyzed/lipemic
	12-0224	9.2	9.4	**9.3**	plasma slightly hemolyzed/lipemic
	12-0253	10.0	9.3	**9.7**	plasma slightly hemolyzed/lipemic
	12-0275	9.3	9.3	**9.3**	plasma slightly lipemic
	12-0293	9.8	9.4	**9.6**	plasma slightly hemolyzed/lipemic
	12-0296	9.7	6.4	**8.1**	plasma slightly hemolyzed/lipemic
	12-0345	10.2	10.0	**10.1**	plasma slightly hemolyzed/lipemic
	12-0354	9.5	9.9	**9.7**	plasma lipemic
	Mean	**9.69**	**9.21**	**9.45**	
	SD	**0.331**	**1.080**	**0.597**	
500 mg/kg	12-0177	9.4	9.2	**9.3**	plasma slightly hemolyzed/lipemic
	12-0189	9.9	9.6	**9.8**	plasma slightly hemolyzed/lipemic
	12-0203	10.8	10.7	**10.8**	plasma slightly hemolyzed/lipemic
	12-0209	9.9	9.4	**9.7**	plasma slightly hemolyzed/lipemic
	12-0236	8.8	9.6	**9.2**	plasma slightly hemolyzed/lipemic
	12-0256	9.7	10.3	**10.0**	plasma slightly hemolyzed/lipemic
	12-0297	9.1	9.5	**9.3**	plasma slightly hemolyzed/lipemic
	12-0314	8.7	9.6	**9.2**	plasma slightly hemolyzed/lipemic
	12-0322	10.5	10.3	**10.4**	plasma slightly hemolyzed/lipemic
	12-0341	9.2	9.4	**9.3**	plasma lipemic
	Mean	**9.60**	**9.76**	**9.68**	
	SD	**0.694**	**0.493**	**0.549**	

(f) = Animal died on study

Table L-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Prothrombin Time Results
Female Rats

Dose	Animal ID	PT Prothrombin Time (sec) Channel A	Channel B	Average	NOTES
Control	12-0211	8.6	9.0	**8.8**	plasma slightly lipemic
	12-0220	8.9	8.7	**8.8**	plasma lipemic
	12-0222	9.2	8.5	**8.9**	plasma slightly hemolyzed
	12-0260	13.9	15.2	**14.6**	plasma lipemic
	12-0289	8.3	8.3	**8.3**	plasma normal
	12-0298[a,c]				
	12-0306	9.4	9.3	**9.4**	plasma normal
	12-0309	8.5	8.2	**8.4**	plasma normal
	12-0317[b]				
	12-0339	9.4	9.2	**9.3**	plasma normal
	Mean	**9.53**	**9.55**	**9.54**	
	SD	**1.816**	**2.318**	**2.060**	
31.25 mg/kg	12-0168	9.4	9.4	**9.4**	plasma slightly lipemic
	12-0170	8.6	8.7	**8.7**	plasma normal
	12-0201[c]	8.9	9.3	**9.1**	plasma hemolyzed
	12-0221	9.3	9.2	**9.3**	plasma normal
	12-0240	9.3	9.2	**9.3**	plasma slightly lipemic
	12-0299[a]				
	12-0300	8.5	8.4	**8.5**	plasma normal
	12-0346	9.6	9.7	**9.7**	plasma slightly hemolyzed/lipemic
	12-0367	9.6	9.3	**9.5**	plasma slightly hemolyzed/lipemic
	12-0369	9.0	9.1	**9.1**	plasma normal
	Mean	**9.13**	**9.14**	**9.14**	
	SD	**0.406**	**0.384**	**0.383**	
125 mg/kg	12-0169	9.3	9.0	**9.2**	plasma normal
	12-0210	8.9	9.2	**9.1**	plasma slightly lipemic
	12-0212	9.1	8.9	**9.0**	plasma lipemic
	12-0227	8.9	8.6	**8.8**	plasma lipemic
	12-0257	8.9	9.2	**9.1**	plasma lipemic
	12-0259	9.3	8.8	**9.1**	plasma slightly lipemic
	12-0261	9.1	8.3	**8.7**	plasma lipemic
	12-0278	8.7	8.3	**8.5**	plasma slightly lipemic
	12-0291	9.7	9.7	**9.7**	plasma normal
	12-0327	9.3	9.9	**9.6**	plasma normal
	Mean	**9.12**	**8.99**	**9.06**	
	SD	**0.290**	**0.534**	**0.373**	
500 mg/kg	12-0166	9.3	9.4	**9.4**	plasma normal
	12-0167	9.1	9.3	**9.2**	plasma normal
	12-0171[a]				
	12-0198	8.8	8.5	**8.7**	plasma slightly lipemic
	12-0229[a]				
	12-0258	10.0	9.1	**9.6**	plasma slightly lipemic
	12-0279	9.1	8.8	**9.0**	plasma lipemic
	12-0308[c]	9.2	12.1	**10.7**	plasma lipemic
	12-0336	10.0	8.9	**9.5**	plasma normal
	12-0348	9.0	9.5	**9.3**	plasma lipemic
	Mean	**9.31**	**9.45**	**9.38**	
	SD	**0.449**	**1.121**	**0.587**	

a= Sperm plug was found but dam was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but dam was pregnant.
d= Animal was not fasted prior to necropsy.

Table L-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Prothrombin Time Results
Recovery Male Rats

		PT			
	Animal	Prothrombin Time (sec)			
Dose	ID	Channel A	Channel B	Average	NOTES
Control	12-0174	10.4	10.2	**10.3**	plasma normal
	12-0179	9.6	9.2	**9.4**	plasma normal
	12-0194	9.3	9.6	**9.5**	plasma normal
	12-0208	8.7	8.7	**8.7**	plasma normal
	12-0272	9.6	9.6	**9.6**	plasma normal
	12-0294	9.3	9.6	**9.5**	plasma normal
	12-0304	9.7	9.5	**9.6**	plasma normal
	12-0323	10.0	9.7	**9.9**	plasma normal
	12-0332	9.5	9.8	**9.7**	plasma normal
	12-0365	9.6	10.0	**9.8**	plasma normal
	Mean	**9.57**	**9.59**	**9.58**	
	SD	**0.447**	**0.415**	**0.406**	
500 mg/kg	12-0176	9.8	9.5	**9.7**	plasma normal
	12-0186	9.5	9.4	**9.5**	plasma normal
	12-0215	11.2	9.7	**10.5**	plasma normal
	12-0244	9.6	9.4	**9.5**	plasma normal
	12-0283	8.5	8.5	**8.5**	plasma normal
	12-0295	9.4	9.7	**9.6**	plasma normal
	12-0302	9.0	8.9	**9.0**	plasma normal
	12-0310	9.5	9.5	**9.5**	plasma normal
	12-0333	9.3	9.8	**9.6**	plasma normal
	12-0363	10.4	10.3	**10.4**	plasma normal
	Mean	**9.62**	**9.47**	**9.55**	
	SD	**0.742**	**0.492**	**0.571**	

Table L-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Summary of Prothrombin Times

Male Rats

		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	NTO in Corn Oil 125 mg/kg	NTO in Corn Oil 500 mg/kg
Average PT	Mean	9.83	9.70	9.45	9.68
	S.D.	0.687	0.656	0.597	0.549
	N	10	9	10	10

Female Rats

		Corn Oil Control	NTO in Corn Oil 31.25 mg/kg	NTO in Corn Oil 125 mg/kg	NTO in Corn Oil 500 mg/kg
Average PT	Mean	9.54	9.14	9.06	9.38
	S.D.	2.060	0.383	0.373	0.587
	N	8	9	10	8

Recovery Male Rats

		Corn Oil Control	NTO in Corn Oil 500 mg/kg
Average PT	Mean	9.58	9.55
	S.D.	0.406	0.571
	N	10	10

Appendix M

Reproductive/Developmental Data

Table M-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Litter Information

		DAY 0 OR 1 LITTER OBSERVATIONS								DAY 4 OBSERVATIONS				
GROUP	DAM ID	POSTPARTUM DAY 0	# GESTATION DAYS	# PUPS/LITTER ALIVE	# PUPS/LITTER DEAD	LIVE LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS	# PUPS/ LITTER	LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS
CONTROL	12-0220	6/19/2012	22	15	1	87.2	8	7	1 stillborn male	15	124.8	6	9	
	12-0339	6/15/2012	22	12	0	56.9	7	5		0				[b]
	12-0309	6/15/2012	22	15	5	89.7	5	10	5 stillborn males	15	131.4	5	10	
	12-0298	n/a												
	12-0260	6/13/2012	22	14	1	75.1	5	9	1 stillborn male[a]	0				
	12-0222	6/16/2012	22	13	0	83.4	7	6		13	131.5	7	6	
	12-0211	6/16/2012	22	8	0	55.5	2	6		8	91.7	2	6	
	12-0317	n/a												
	12-0289	6/15/2012	22	17	0	99.4	9	8		17	146.8	9	8	
	12-0306	6/14/2012	22	8	7	46.6	4	4	2 stillborn males 5 stillborn females	8	82.4	4	4	
		Mean	**22**	**12.8**	**1.8**	**74.23**	**5.9**	**6.9**		**9.5**	**118.10**	**5.5**	**7.2**	
		S.D.	**0**	**3.28**	**2.71**	**19.051**	**2.30**	**2.03**		**6.70**	**25.284**	**2.43**	**2.23**	

a= Litter was euthanized on 6/15/12 due to complete neglect by dam. Pups were cold with no milk visible in their stomachs. No visible abnormalities.
13 pups remaining at time of euthanasia with litter weight of 62.2 grams.
b= 3 of the pups were found dead on 6/17/12 and all other pups were consumed by dam.

Table M-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Litter Information

		DAY 0 OR 1 LITTER OBSERVATIONS								DAY 4 OBSERVATIONS				
GROUP	DAM ID	POSTPARTUM DAY 0	# GESTATION DAYS	# PUPS/LITTER ALIVE	# PUPS/LITTER DEAD	LIVE LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS	# PUPS/ LITTER	LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS
31.25	12-0300	6/15/2012	22	10	0	74.9	6	4		10	115.9	6	4	
mg/kg	12-0168	6/13/2012	22	12	1	85.6	7	5	1 stillborn male 1 male runt	12	122.7	7	5	1 male runt
	12-0240	6/13/2012	22	12	0	77.5	5	7		12	113	5	7	
	12-0367	6/14/2012	22	12	0	69.4	8	4		12	110.1	8	4	
	12-0170	6/17/2012	23	17	0	106.7	6	11	2 male runts[a]	15	150.4	5	10	1 male runt[b]
	12-0221	6/26/2012	22	15	1	95.1	7	8	1 stillborn female 2 males w/abrasions	15	123.7	7	8	
	12-0299	n/a												
	12-0201	6/19/2012	Unknown	15	0	83.4	8	7		15	133.6	8	7	
	12-0369	6/16/2012	22	16	0	96.1	13	3		15	136.3	13	2	[b]
	12-0346	6/14/2012	22	12	0	76.7	6	6		12	119.4	6	6	
		Mean	**22.1**	**13.4**	**0.2**	**85.04**	**7.3**	**6.1**		**13.1**	**125.01**	**7.2**	**5.9**	
		S.D.	**0.35**	**2.35**	**0.44**	**12.091**	**2.35**	**2.47**		**1.90**	**12.913**	**2.44**	**2.42**	

a= 1 male pup was discovered dead on 6/19/12 weighing 3.6 grams.
b= 1 female pup was not found and was likely consumed by dam.

Table M-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Litter Information

		DAY 0 OR 1 LITTER OBSERVATIONS								DAY 4 OBSERVATIONS				
GROUP	DAM ID	POSTPARTUM DAY 0	# GESTATION DAYS	# PUPS/LITTER ALIVE	# PUPS/LITTER DEAD	LIVE LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS	# PUPS/ LITTER	LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS
125	12-0212	6/15/2012	22	12	0	78.9	5	7		12	117.6	5	7	
mg/kg	12-0169	6/16/2012	23	15	2	77.3	10	5	2 stillborn males	0				[a]
	12-0257	6/15/2012	22	14	0	83.6	7	7		14	132.2	7	7	1 female runt
	12-0227	6/14/2012	22	15	0	98.6	9	6		14	132.6	8	6	
	12-0210	6/16/2012	22	7	0	49.4	2	5		6	72.9	2	4	[b]
	12-0291	6/17/2012	22	14	0	91.4	7	7		14	122.6	7	7	
	12-0278	6/16/2012	21	17	0	103.7	8	9		17	151	8	9	
	12-0261	6/14/2012	22	15	0	90.5	10	5		15	141.3	10	5	
	12-0259	6/17/2012	22	15	1	76.4	11	4	1 stillborn male	0				[c]
	12-0327	6/17/2012	22	10	1	71.4	6	4	1 stillborn male	10	98.9	6	4	
		Mean	**22.0**	**13.4**	**0.4**	**82.12**	**7.5**	**5.9**		**10.2**	**121.14**	**6.6**	**6.1**	
		S.D.	**0.47**	**2.95**	**0.70**	**15.448**	**2.72**	**1.60**		**6.16**	**25.037**	**2.39**	**1.73**	

a= 8 dead pups and 5 live pups were found on postpartum day 1. 2 pups were missing and likely consumed by dam.
Remaining 5 pups were missing and likely consumed by dam on postpartum day 2.
b= 1 female pup was missing on postpartum day 4 and was likely consumed by dam.
c= Entire litter was abandoned by dam. Pups were not nursing and were cold. All 15 pups euthanized on 6/18/12 with a litter weight of 74.0 grams.

Table M-4
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Litter Information

		DAY 0 OR 1 LITTER OBSERVATIONS								DAY 4 OBSERVATIONS				
GROUP	DAM ID	POSTPARTUM DAY 0	# GESTATION DAYS	# PUPS/LITTER ALIVE	# PUPS/LITTER DEAD	LIVE LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS	# PUPS/ LITTER	LITTER WEIGHT (grams)	# MALES	# FEMALES	OBSERVATIONS
500 mg/kg	12-0167	6/20/2012	22	12	1	80.8	7	5	2 male runts 1 stillborn male	11	109	5	6	1 male runt [c]
	12-0308	6/15/2012	Unknown	10	0	76	4	6		10	136	4	6	
	12-0336	6/13/2012	22	11	0	70.6	6	5		11	111.4	6	5	
	12-0348	6/14/2012	22	15	0	95.2	8	7	1 female runt	14	132	8	6	[d]
	12-0171	n/a												
	12-0279	6/14/2012	22	16	0	83.1	6	10		15	112.6	6	9	1 male/1 female ru
	12-0229	n/a												
	12-0198	6/15/2012	22	14	0	89.3	11	3		14	145.9	11	3	
	12-0258	6/14/2012	22	15	2	72.2	8	7	2 stillborn males	0				[a]
	12-0166	6/17/2012	22	14	0	79.4	4	10	4 female runts 1 male runt	13	122.9	3	10	3 female runts [b]
		Mean	**22.0**	**13.4**	**0.4**	**80.83**	**6.8**	**6.6**		**11.0**	**124.26**	**6.1**	**6.4**	
		S.D.	**0.00**	**2.13**	**0.74**	**8.349**	**2.31**	**2.45**		**4.78**	**14.154**	**2.67**	**2.37**	

a= Entire litter was abandoned by dam. Pups were not nursing and were cold. All 15 pups euthanized on 6/15/12 with a litter weight of 66.3 grams. Remaining 5 pups were missing and likely consumed by dam on postpartum day 2.

b= 1 male pup was found dead on 6/18/12 weighing 3.7 grams.

c= 1 of the male runts was missing and likely consumed by dam.

d= 1 female runt was missing and likely consumed by dam.

Table M-5
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Reproductive/Developmental Tabular Summary Table

OBSERVATIONS	VALUES			
	Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Pairs started (N)	10	10	10	10
Females showing evidence of copulation (N)	9	9	10	9
Females achieving pregnancy (N)	8	9	10	8
Conceiving days 1-5 (N)	8	8	10	7
Conceiving days 6-13 (N)	1	1	0	2
Pregnancy = 21 days (N)	0	0	1	0
Pregnancy = 22 days (N)	8	7	8	7
Pregnancy = 23 days (N)	0	1	1	0
Unknown (sperm plug not found)	0	1	0	1
Dams with live young born (N)	8	9	10	8
Dams with live young at day 4 pp (N)	6	9	9	7
Corpora lutea/dam (mean)	17.8	16.1	16.6	19.8
Implants/dam (mean)	15.3	15.6	15.5	15.3
Live pups/dam at birth (mean)	12.8	13.4	13.4	13.4
Live pups/dam at day 4 (mean)	12.7	13.1	13	12.6
Sex ratio (m/f) at birth (mean) (live pups)	0.9	1.5	1.38	1.28
Sex ratio (m/f) at day 4 (mean) (live pups)	0.8	1.74	1.3	1.24
Litter weight at birth (mean) (live pups)	74.23	85.04	82.12	80.8
Litter weight at day 4 (mean) (live pups)	118.1	125.01	115.9	124.3
ABNORMAL PUPS (including stillbirths)				
Day 0				
Dams with 0	4	6	7	4
Dams with 1	2	1	2	1
Dams with 2		2	1	1
Dams with 3				1
Dams with 4				
Dams with 5+	2			1
LOSS OF OFFSPRING				
Pre-implantation (CL's - implantations)				
Females with 0	4	5	7	4
Females with 1	1	3	1	
Females with 2		1		1
Females with 3	1			
Females with 4	1		1	
Females with 5+	1		1	3
Pre-natal (implantations - live births)				
Females with 0	2	2	2	3
Females with 1	2	1	3	
Females with 2	1	1	3	4
Females with 3	1	4	1	
Females with 4		1		
Females with 5+	2		1	1
Post-natal (live births - alive at post natal day 4)*				
Females with 0	6	7	7	3
Females with 1		1	2	4
Females with 2		1		
Females with 3				
Females with 4				
Females with 5+	2		1	1

*Pups/litters that were humanely euthanized due to total neglect included as post-natal deaths

Appendix N

Gross Pathology Data

Table N-1
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Gross Necropsy Findings
Male Rats

Group	Animal ID	Gross Findings
Corn Oil Control	12-0163	Mildly pale liver
	12-0205	Dark red liver; Mildly dark spleen
	12-0206	Pale Intestine
	12-0207	Mildly pale intestine with yellow fluid throughout; Dark kidneys
	12-0235	None
	12-0254	Very small left testes
	12-0263	Slightly pale intestine
	12-0267	Mildly enlarged, dark spleen; Dark kidneys
	12-0284	Dark red liver; Dark kidneys; Enlarged, dark spleen; Pale small intestine with bright yellow fluid throughout
	12-0313	Dark liver; Pale small intestine with bright yellow fluid
31.25 mg/kg	12-0164	Dark red liver; 2 pale brown 1 mm areas on left lobe of thymus
	12-0165	Mildly pale liver; Yellow material in stomach
	12-0225	Mildly pale liver; Pale small intestine with bright yellow material throughout
	12-0234	Pale small intestine with bright yellow fluid throughout
	12-0243	Bright yellow material in small intestine
	12-0245	Dark red liver; Dark kidneys; Pale small intestine with bright yellow fluid throughout
	12-0255	Dark kidneys; Mildly dark spleen; Pale small intestine with bright yellow fluid throughout
	12-0262	Mildly dark liver
	12-0324	Dark liver; Dark spleen
	12-0351	Found dead on 5/25/12
125 mg/kg	12-0187	Pale small intestine with bright yellow material throughout
	12-0204	Mildly dark spleen; Mottled red liver; Pale small intestine with bright yellow fluid throughout
	12-0223	Pale small intestine with bright yellow material throughout
	12-0224	Pale intestine with bright yellow fluid throughout
	12-0253	Dark red liver; Dark kidneys; Pale small intestine with pale yellow fluid throughout
	12-0275	Dark liver; Pale small intestine with bright yellow fluid throughout
	12-0293	Mildly dark kidneys
	12-0296	Pale small intestine with bright yellow material throughout
	12-0345	Dark liver; Slightly small spleen; Dark kidneys
	12-0354	Pale small intestine with bright yellow material throughout
500 mg/kg	12-0177	Small testes; Mildly enlarged spleen; Pale intestine with bright yellow material throughout
	12-0189	Small testes; Dark red liver
	12-0203	Small testes; Pale small intestine with bright yellow material throughout
	12-0209	Small testes; Multi-focally red mottled liver; Bright yellow fluid in small intestine
	12-0236	Small testes; Dark red liver; Dark kidneys; Pale small intestine with bright yellow fluid throughout
	12-0256	Small testes; Dark liver; Dark kidneys; Pale small intestine with bright yellow fluid throughout
	12-0297	Small testes; Mildly dark liver; Enlarged dark spleen; Pale intestine with bright yellow material throughout
	12-0314	Small testes; Dark liver; Pale red spleen; Pale intestine with bright yellow material throughout
	12-0322	Small testes
	12-0341	Small testes; Dark liver; Dark kidneys; Pale small intestine with bright yellow fluid throughout

Table N-2
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Gross Necropsy Findings
Female Rats

Group	Animal ID	Gross Findings
Corn Oil Control	12-0211	Dark liver; Mildly dark kidneys; Mildly dark spleen
	12-0220	Yellow fluid in intestine
	12-0222	Mildly dark liver
	12-0260	7 x 5 mm scab on ventral chin; Pale liver
	12-0289	Multiple red spots on lower lobe of lung
	12-0298[a]	Pale small intestine
	12-0306	Dark kidneys; Pale small intestine
	12-0309	None
	12-0317[b]	Dark liver; Pale intestine; 9 x 4 mm "diverticulum" on serosal side of greater curvature of stomach. No mucosal defect noted. Musculature appears to be distending out from defect.
	12-0339	Bright yellow fluid throughout intestine; Both ovaries have fluid-filled cysts
31.25 mg/kg	12-0168	Pale small intestine with bright yellow fluid present; Yellow-tinged mesenteric lymph nodes
	12-0170	Pale small intestine
	12-0201[c]	5 x 2 mm mass in fat near ovary; Yellow fluid in intestine
	12-0221	Yellow liquid in stomach and intestine
	12-0240	Dark liver; Pale small intestine with small amount of bright yellow fluid present
	12-0299[a]	Pale small intestine
	12-0300	Bright yellow fluid throughout intestine
	12-0346	Mildly enlarged submandibular lymph nodes; Pale small intestine
	12-0367	Pale small intestine with bright yellow fluid present
	12-0369	None
125 mg/kg	12-0169	Yellow fluid in small intestine
	12-0210	Mildly red mottled lungs; Mildly dark liver; Bright yellow fluid in intestine
	12-0212	Bright yellow fluid throughout intestine
	12-0227	Pale intestine
	12-0257	Bright yellow fluid throughout intestine
	12-0259	Yellow-tinged subcutaneous brown fat; Pale, brown liver; Mottled red lungs; Mildly dark kidneys; Thin with minimal visceral fat
	12-0261	Mildly distended stomach with bedding; Pale small intestine
	12-0278	Mildly enlarged submandibular lymph nodes; Mildly dark liver; Pale intestine
	12-0291	Pale small intestine
	12-0327	Dark liver; Right kidney cystic and enlarged; Left kidney cystic
500 mg/kg	12-0166	Mildly dark liver and spleen
	12-0167	Yellow fluid in stomach; Pale intestine
	12-0171[a]	Yellow fluid in intestine
	12-0198	Yellow fluid throughout intestine
	12-0229[a]	Mildly fluid-filled uterus
	12-0258	Dilated uterus; Mildly dark kidneys; Bright yellow fluid in small intestine
	12-0279	Pale yellow staining of fur on abdomen and vulva; Mildly gas-distended cecum
	12-0308[c]	Yellow fluid throughout intestine
	12-0336	Small amount of fluid in small intestine
	12-0348	Mildly distended stomach with bedding; Pale small intestine

a= Sperm plug was found but female was not pregnant.
b= No sperm plug found and was not pregnant.
c= No sperm plug found but female was pregnant.

Table N-3
Protocol No. 0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental
Toxicity of NTO in the Rat

Individual Gross Necropsy Findings
Recovery Male Rats

Group	Animal ID	Gross Findings
Corn Oil Control	12-0174	Mildly enlarged sub-mandibular lymph nodes; Mildly dark liver
	12-0179	Mildly dark liver; Mildly dark spleen
	12-0194	Dark spleen; Dark kidneys; Hydronephrosis of right kidney
	12-0208	Dark liver
	12-0272	None
	12-0294	Dark liver; Dark spleen
	12-0304	Dark liver
	12-0323	None
	12-0332	Dark liver
	12-0365	None
500 mg/kg	12-0176	Small testes; Mildly enlarged, dark spleen
	12-0186	Dark liver
	12-0215	Small testes; Dark, enlarged spleen, Dark kidneys
	12-0244	Small testes
	12-0283	Small testes; Dark liver
	12-0295	Small right testes
	12-0302	Dark liver; Mildly dark, mottled kidneys
	12-0310	Small testes; Dark liver; Dark, mottled kidneys
	12-0333	Small testes; Dark liver; Hydronephrosis of right kidney
	12-0363	Small testes; Dark liver

Appendix O

Histopathology Report

Pathology report for
0FP4-93-12-03-03
Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

11 JULY 2013

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2. INTRODUCTION

As a result of an initiative by the Department of Defense (DOD) to improve munitions safety, the US Army is developing insensitive munitions (IM) for incorporation into its inventory of conventional military munitions systems. Despite the slightly lower performance of NTO compared to TNT, there has been a renewed interest in NTO use in explosive formulations based on its lower sensitivity as a melt-cast medium observed during testing and the less stringent shipping requirements. To support possible fielding of these PAX explosives, additional reproductive/developmental toxicity data in a mammalian system needs to be generated to assess the occupational health hazards associated with the use and production of this material.

The primary objective of this study was to determine the initial reproductive and developmental toxicity of NTO through the use of a screening test. The secondary objective of this study was to confirm the effects of repeated-dose exposure to NTO using different exposure durations and dose levels than previously evaluated.

3. METHODS

Animals were oral gavaged with NTO suspended/dissolved in corn oil. Forty adult Sprague Dawley rats of each sex (N=80) were randomly distributed into 3 dose groups, 31.25 mg/kg, 125 mg/kg, 500 mg/kg and a vehicle control group (10 rats of each sex per dose group). Rats were exposed daily to control, 31.25, 125, and 500 mg/kg-day NTO 7 days/week for a maximum of 56 days.

In addition to the main study, 10 male rats per group were added to serve as a satellite recovery groups for the highest dose group and the control group. These animals were dosed concurrently with the main study animals for the appropriate time period and held for a period of at least 14 days (not to exceed one month) following cessation of dosing. Animals used as satellite/recovery animals were not mated but were anesthetized, bled, and necropsied with selected tissues sent for histopathological evaluation. The purpose of the satellite group is to evaluate the reversibility, persistence, or delayed occurrence of toxic effects associated with repeated exposure to NTO.

Dosing of both sexes began 2 weeks prior to mating following an acclimatization period of no less than 5 days. Dosing was continued in both sexes during the 2 week mating period. Male rats were dosed with NTO for the minimum dosing period of 28 days. Following the 28-day dosing period, male rats were anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation. Female rats with no evidence of copulation (presence of sperm plug) following the 2-week mating period were dosed and euthanized 24-26 days after the last day of the mating period. These animals were anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation. Daily dosing of the parental females were continued throughout pregnancy and at least up to, and including, day 3 post-partum or the day before sacrifice. However, in order to allow for overnight fasting of dams prior to blood collection, the pups were euthanized on post-partum day 4 with euthanasia of the dams occurring the next day. Gross external examinations of all pups occurred on the day they were euthanized. Parental females

were also anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation.

All adult animals in the study were subjected to a full, detailed gross necropsy including careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Special attention was paid to the organs of the reproductive system. All gross pathology changes were recorded on CHPPM Form 402-R-E. The following organs and tissues, or representative samples, were preserved in a suitable medium for histopathological examination: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid parathyroid; thymus; lungs and trachea; pharynx; larynx; nose; heart; bone marrow (either femur, sternum or rib at the costochondral junction); salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; trachea; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar) and exorbital lachrymal glands. In addition, the following organs were weighed: liver, kidneys, adrenals, gonads, spleen, brain, epididymides, uterus, thymus and heart. Prior to being weighed, organs were carefully dissected and trimmed to remove fat and other tissue in a uniform manner. All routine tissues were preserved in formalin fixative; testes and epididymis were preserved in Modified Davidson's fixative. Unless otherwise, individually specified, left epididymis and left testis were evaluated for each male. In the 500mg/kg recovery group, both right and left testis were generally examined.

All tissues were selectively trimmed and placed in cassettes labeled with protocol number, animal identification number and laboratory assigned accession number. Cassettes were placed in labeled formalin or Modified Davidson's 70% filled containers, and transported to the US Army Institute of Chemical Defense (USAMRICD) for processing. Tissues were routinely processed, paraffin embedded, microtomed at 5μm and stained routinely with routine hematoxylin and eosin.

The pathologist examined slides for compound-induced histopathologic changes via light microscopy. The prevelance and severity of findings were graded as compared to controls. Control animals were examined for background findings and all findings were recorded. Findings, in all animals and groups, were assigned as none, minimal, mild, moderate or severe.

4. RESULTS

4.1 Clinical Pathology

For the main study group males, statistically significant differences between dose groups were only present for total bilirubin (TBIL) and inorganic phosphorus (PHOS). For TBIL, the 500 mg/kg dose group had a significantly higher value compared to the 125 mg/kg dose group. For PHOS, even though the p-value was less than .05, the post-hoc test found no significant differences between dose groups after adjusting for

multiple comparisons. Increased TBIL can be associated with hemolysis and liver pathology. In sections of liver examined, there were no significant hepatocellular or biliary system findings that would indicate a cause for this increased value. The cause and significance of the TBIL increase is undetermined.

In the male recovery group, there were no significant differences found between the control group and 500 mg/kg dose group averages for any of the seventeen clinical chemistry measures.

The only significant difference in female clinical chemistry results was in inorganic phosphorus (PHOS) where the 500 mg/kg dose had a higher mean PHOS level than the 125 mg/kg dose. Increased phosphorous can be due to renal failure, hemolysis, hypervitaminosis D, hypoparathyroidism, or osteolytic bone lesions. There were no additional clinical pathology results in support of these possible differential causes, the cause and significance of the increased phosphorous in this group is undetermined.

4.2 Gross necropsy findings

Dark kidneys and dark spleens were noted equivalently across all groups. There was no correlated histologic finding. This varied gross color observation is likely due to blood congestion and is not treatment related. Pale small intestines, in male rats, were recorded in 5/10 controls, 4/9 of 31.25 mg/kg, 8/10 of 125 mg/kg, and 8/10 of 500 mg/kg. In pregnant female rats, pale small intestines were recorded in 1/8 controls, 4/9 of 31.25 mg/kg, 1/10 of 125 mg/kg and 2/9 of 500 mg/kg. This observation was likely due to oral corn oil administration. This was confirmed microscopically, appearing as microvacuolation of apical enterocytes. This finding is not compound treatment related.

4.3 Gross necropsy/microscopic finding correlating to litter observations

Animal #-0259 in the 125 mg/kg group was observed with 1 dead pup on post-partumday 0 and the remaining pups were euthanized on post-partum day 1 due to neglect by the dam. Gross necropsy observations in this female were an overall thin body condition with yellow tinged subcutaneous brown fat, minimal visceral fat, pale brown liver, mottled red lungs and mildly dark kidneys. Microscopic findings included renal tubular vacuolation, interpreted as tubular degeneration, hepatocellular glycogen accumulation and apoptosis and severe thymic atrophy. Renal and hepatic findings can be related to a primary disease process, however, they can also be due severe physiological stress, and a high catabolic state such as pregnancy which can compromise organ function. This female's general compromised and ill state may have contributed to the abandonment of the pups. It is undetermined if the NTO exposure contributed to findings. Of the other female rats noted to have dead or runt pups or euthanasias due to neglect, there were no significant correlative microscopic findings to explain the litter observations.

4.4 Microscopic findings

Hepatic lymphocytic infiltration was noted in all males and females, all dose groups, as well as controls. Few isolated aggregates of mononuclear cells can be considered a background lesion, however, severity of this lesion may intensify with chemical

exposures. (Thoolen 2010) Increase in dosage did not affect the frequency of this lesion. It was, therefore, considered a background finding.

Main Study Males
Extramedullary hematopoesis was noted in the majority of males in all treatment groups. The incidence and severity was observed at a similar rate across all treated groups, did not increase with dose, and is, therefore, not treatment related.

Table 1. Incidence and severity of Splenic EMH Males- Main Study				
Dose group	Control	31.25 mg/kg	125 mg/kg	500 mg/kg
None	0/10	2/10	0/10	0/10
Minimal EMH	5/10	3/10	1/10	3/10
Mild EMH	5/10	4/10	9/10	7/10
Moderate EMH		1/10		
Severe EMH				

Test article-related lesions were present in the testes and epididymis of all 10/10 500 mg/kg rats. The majority of testicular seminiferous tubules were shrunken, retaining only Sertoli cells, spermatogonia and leptotene and zygotene spermatocytes. Few tubules retained pachytene spermatocytes, however, most were degenerate. Severe degeneration/atrophy of the left testis was observed in control animal 12-0254. This finding is considered to be congenital; an underdeveloped testis and not related to the study.

Table 2. Incidence and severity of Testicular lesions- Main Study				
Dose group	Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Normal	9/10	10/10	10/10	
Minimal tubular degeneration/atrophy				
Mild tubular degeneration/atrophy				
Moderate tubular degeneration/atrophy				
Severe tubular degeneration/atrophy	1/10			10/10

Moderate or complete absence of sperm was observed in all 500 mg/kg males. Moderate hypospermia was defined as absence of mature spermatids in the head and body of the epididymis with mature spermatids evident in the tail section. Mature spermatids were not found in any epididymal segment in severe hypospermia/aspermia animals.

Cribiform change of the epididymis was observed in 10/10 500 mg/kg rats of minimal and mild severity. Control rat #0254 was noted to have moderate cribiform change. Cribiform change is an infolding and bridging of the epithelium in segments of ducts that have undergone contraction. It is often seen in the distal corpus/proximal caput in association with decreased/absent sperm and ductular atrophy.(Creasy 2012)

Table 3. Incidence and severity of Epididymal lesions- Main Study

Dose group	Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Normal	9/10	10/10	10/10	
Mild hypospermia				
Moderate hyposerpermia				3/10
Severe hypospermia/aspermia	1/10			7/10

Recovery Males

Complete recovery was not evident in these males. All spermatogonia and spermatocytes were present through all stages. Descriptively, tubules were graded as follows:

Mild degeneration/atrophy was defined as: Variable mature 13-18 and 19 spermatids missing.

Moderate degeneration/atrophy defined as: All spermatogonia and spermatocytes present through all stages. Spermatids 1-11/12 generally present with some 7-10 spermatid loss 13-18 variably present. No mature 19s.

Severe degeneration/atrophy observed: by stage, I-V completely intact. Stages VII-VIII missing spermatid 19 and stages IX-XIV missing 9-14 spermatids with more completely atrophic tubules.

Table 4. Incidence and severity of Testicular lesions-Recovery			
Dose group	Control	500 mg/kg	
	Left	Left	Right
Normal	10/10	0/10	0/8
Minimal tubular degeneration/atrophy		0/10	0/8
Mild tubular degeneration/atrophy		4/10	4/8
Moderate tubular degeneration/atrophy		4/10	3/8
Severe tubular degeneration/atrophy		2/10	1/8

One male was excluded from the incidence rate for hypospermia due to the absence of the tail in sections examined.

Table 5. Incidence and severity of Epididymal lesions- Recovery		
Dose group	Control	500mg/kg
Normal	10/10	
Mild hypospermia		
Moderate hypospermia		7/9
Severe hypospermia/aspermia		2/9

Main Study Females

Microscopically, a decrease in cortical lymphocytes was observed in pregnant controls and dose groups. In pregnant females, an early increase in thymic weight is followed by a marked reduction in cellularity of the cortex. The level of cellularity returns to normal once pregnancy is over. Increased levels of progesterone during pregnancy have a negative effect on thymic weight, whereas increased prolactin occurring during lactation has a stimulatory effect on the thymus.(Pearse 2006) Although thymic cortical cellularity decreased, weight did not change across pregnant animals so this microscopic finding was considered to be associated with pregnancy and not treatment related.

Table 6. Incidence of Thymic findings Pregnant Females

Dose group	Control	31.25mg/kg	125mg/kg	500mg/kg
None	3/8	6/9	5/10	5/7
Cortical lymphocyte loss/increased medullary lymphyocytes	5/8	3/9	5/10	2/7

Extramedullary hematopoeisis was noted in all pregnant females. The incidence and severity was observed at a similar rate across all treated groups, did not increase with dose and therefore, no dose effect was observed.

Table 7. Incidence and severity of Splenic EMH Pregnant Females- Main Study				
Dose group	Control	31.25mg/kg	125mg/kg	500mg/kg
None				
Minimal EMH	2/8	3/9	2/10	2/8
Mild EMH	2/8	3/9	4/10	5/8
Moderate EMH	4/8	3/9	4/10	1/8
Severe EMH				

5. DISCUSSION

Testicular toxicants can target multiple sites within the male reproductive system. Toxicants can act directly on testicular blood supply and cells (leydig cells, sertoli cells, spermatogonia, spermatocytes, spertmatids, spermatozoa) or at extratesticular sites (hypothalamus-pituitary axis and central nervous system) resulting in direct damage to those cells or those cells they physiologically support.

Repetitive and prolonged dosing, regardless of the mechanism of toxicity, will result in germ cell damage and loss. Germ cells are affected because they are dependent on the function and processes of other cell types within the testis; a disruption of the germ cell supporting environment often results in their death (Creasy 1997). Since progressive germ cell loss occurs throughout a repeat dose, long term study, the end result is often seminiferous tubules lined only by Sertoli cells. Even though Sertoli cells are sensitive to alterations in function, they are extremely resistant to cell death (Creasy 2001).

In all 500mg/kg main study males, severe tubular degeneration/atrophy was observed, with retention of Sertoli cells and minimal retention of all first layer germ cells.

If it is necessary to elucidate the target cell, a time course study should be performed in order to identify the earliest stage of pathologic change. The most interesting time period then should be chosen for an in-depth analysis (Creasy 1997).

All spermatogonia and spermatocytes were present through all stages for recovery 500 mg/kg males. Maturing spermatids were variably present. Complete recovery was not evident in these males. Recovery periods should be timed as multiples of the spermatogenic process, 8 weeks in rats. In theory, this allows for any reversible injury to spermatogonia at the end of the dosing period to work its way through the maturation depletion and allow full recovery of all cell layers. If recovery is delayed or the recovery period is less than the 6-8 week duration, the testes may show a more severe weight loss and depletion of germ cells in the recovery animals than was present in the terminal kill animals. This is predictable, given the kinetics of cell division and multiplication and the progressive maturation depletion of the descendant cohorts of cells during the recovery period. (Creasy 1997).

6. REFERENCES

Creasy DM (2001). Pathogenesis of male reproductive toxicity. Toxicol Pathol, 29(1): 64-76.

Creasy DM (1997). Evaluation of testicular toxicity in safety evaluation studies: The appropriate use of spermatogenic staging. Toxicol Pathol, 25(2): 119-131.

Creasy D, Bube A, DeRijk E, Kandori H, Kuwahara M, Masson R, Nolte T, Reams R, Regan K, Rehm S, Rogerson P, Whitney K (2013). Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. Toxicol Pathol, 40: 40S-121S.

Foley GL (2001). Overview of male reproductive pathology. Toxicol Pathol, 29(1): 49-63.

Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ, Regan KS, Goodman DG (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicol Pathol, 30(4): 507-520.

Nolte T, Harleman JH, Jahn W (1995). Histopathology of chemically induced testicular atrophy in rats. Exp Toxic Pathol, 47: 267-286.

Pearse, Gail (2006). Histopathology of the Thymus. Toxicol Pathol, 34: 515-547.

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Kuttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Bix AE, Singh B, Belpoggi F, Ward JM (2010) Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol, 38:5S-81S.

7. HISTOPATHOLOGY DATA

MALES

Control

12-0163
1. Kidney, left and right: Infiltrates, mononuclear, interstitial, multifocal, mild.
2. Liver: Infiltrates, mononuclear, multifocal, random, mild.
3. Liver: EMH, focal, minimal.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Spleen: EMH, multifocal, minimal.
5. Small intestine; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Mildly pale liver.

12-0205
1. Kidney, left: Basophilic tubules, multifocal, minimal with rare mononuclear infiltrates.
2. Kidney, right: No significant findings.
3. Liver: Infiltrates, mononuclear, multifocal, random, minimal.
4. Liver: EMH, focal, minimal.
5. Spleen: Hemosiderosis, multifocal, minimal.
6. Spleen: EMH, multifocal, minimal.
7. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Dark, red liver. Mildly dark spleen.

12-0206
1. Kidney, left: Cystic tubule, focal, with fibrosis.
2. Kidney, left: Basophilic tubules, multifocal, minimal with mononuclear interstitial infiltrates.
3. Kidney, right; jejunum; thymus; testis; epididymis: No significant findings.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Spleen: EMH, multifocal, minimal.
6. Liver: Infiltrates, mononuclear, multifocal, random, minimal.
7. Liver: EMH, focal, minimal

Gross necropsy findings: Pale intestines.

12-0207
1. Liver: Vacuolation, micro- and macro, diffuse, moderate.
2. Liver: EMH, focal, minimal.
3. Kidney, right and left; jejunum; thymus; testis; epididymis: No significant findings.
4. Spleen: EMH, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, minimal.

Gross necropsy findings: Mildly pale intestine with bright yellow fluid; stomach has moderate amounts of bedding; Dark kidneys.

12-0235
1. Kidney, right and left: Infiltrates, mononuclear, interstitial, multifocal, minimal.
2. Liver: Infiltrates, mononuclear, multifocal and random, mild.
3. Liver: Vacuolation, micro-, diffuse, moderate.

4. Spleen: EMH, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: None. *Note: right and left kidney each cross cut; unable to distinguish between right and left

12-0254

1. Testis, left: Tubular degeneration/atrophy, diffuse, severe.
2. Epididymis: Aspermia, diffuse, severe with cribiform change and eosinophilic cauda tubular debris.
3. Kidney,left: Cystic tubule, focal, moderate.
4. Spleen: EMH, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, minimal.
6. Liver: Infiltrates, mononuclear, multifocal and random, mild.
7. Liver: EMH, focal, minimal.
8. Liver: Vacuolation, micro-, diffuse, moderate.

Gross necropsy findings: Small left testis.

12-0263

1. Kidney, right and left; small intestine; testis: No significant findings.
2. Liver: Infiltrates, mononuclear, multifocal and random, mild.
3. Liver: Vacuolation, micro-, diffuse, moderate
4. Spleen: EMH, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Epididymis: Infiltrates, mononuclear, interstitial, multifocal, minimal.

Gross necropsy findings: Slightly pale intestine.

12-0267

1. Kidney, left: Basophilic tubules, multifocal, mild with mononuclear infiltrates.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, mild
4. Liver: Vacuolation, micro- and macro-, diffuse, moderate.
5. Liver: Cystic degeneration, focal, minimal.
7. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
6. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Mildly enlarged dark spleen. Dark kidney.

12-0284

1. Kidney, right: Infiltrates, mononuclear, focal, minimal.
2. Spleen: EMH, multifocal, minimal.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Liver: Vacuolation, micro- and macro-, diffuse, moderate.
6. Kidney, left; thymus; small intestine; testis; epididymis: No significant findings.

Gross necropsy findings: Dark, red liver. Dark kidneys. Enlarged dark spleen. Pale small intestine with bright yellow fluid.

12-0313

1. Kidney, left: Infiltrates, mononuclear, multifocal, mild with basophilic tubules.
2. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
3. Liver: Vacuolation, micro-, diffuse, moderate.
4. Spleen: EMH, multifocal, minimal.
5. Spleen: Hemosiderosis, multifocal, mild.
6. Small intestine, enterocytes: Microvacuolation, diffuse, mild.
7. Kidney, right; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Dark liver. Pale small intestine with bright yellow fluid.

500mg/kg

12-0177

1. Kidney, left: Basophilic tubules, focal, mild.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Liver: EMH, rare.
6. Small intestine, enterocytes: Microvaculation, diffuse, severe.
7. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.
8. Epididymis, head, body, tail: Aspermia, diffuse, severe with minimal cellular debris and tubular dilatation.
9. Epididymis, body: Cribiform change, mild.
10. Kidney, right; thymus: No significant findings.

Gross necropsy findings: Small testes. Mildly enlarged spleen. Pale intestine with bright yellow fluid. Missing pituitary.

12-0189

1. Kidney, left: Infiltrates, mononuclear, interstitial, multifocal, mild with rare basophilic tubules.
2. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
3. Liver: Micro- and macrovacuolation, diffuse, moderate.
4. Liver: EMH, focal, minimal.
5. Spleen: EMH, multifocal, mild.
6. Spleen: Hemosiderosis, multifocal, minimal.
7. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.
8. Epididymis, head, body, tail: Aspermia, diffuse, severe with minimal cellulular debris and tubular dilatation.
9. Epididymis, body: Cribiform change, mild.
10. Epididymis, head: Infiltrates, lymphocytic, perivascular, focal, minimal.
12. Kidney, right; thymus: No significant findings.

Gross necropsy findings: Small testes. Dark red liver.

12-0203

1. Kidney, left: Basophilic tubules, focal, minimal.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Liver: Microvacuolation, diffuse, moderate.
6. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe with interstitial eosinophilic amorphous material (fluid).

7. Epididymis, head, body, tail: Aspermia, diffuse, severe with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Thymus: Apoptosis, cortical, multifocal, mild with tingible body macrophages.
10. Kidney, right; small intestine : No significant findings.

Gross necropsy findings: Small testes. Pale small intestine with bright yellow fluid.

12-0209

1. Kidney, left and right: Infiltrates, mononuclear, multifocal, mild with basophilic tubules.
2. Spleen: EMH, multifocal, moderate.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Micro- and macrovacuolation, diffuse, moderate.
5. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe with interstitial eosinophilic amorphous material (fluid).
6. Epididymis, head, body: Hypospermia, multifocal, moderate with minimal tubular dilatation.
7. Epididymis, body: Cribiform change, mild.
8. Epididymis, tail: Cellular debris and mature spermatids.
9. Small intestine, enterocytes: Microvacuolation, diffuse, moderate.
10. Thymus: No significant findings.

Gross necropsy findings: Small testes. Liver multifocal mottled red. Pale small intestine. Bedding in stomach. Bright yellow fluid in small intestine.

12-0236

1. Liver: Micro- and macrovacuolation, diffuse, moderate.
2. Liver: Infiltrates, mononuclear, multifocal and random, mild.
3. Spleen: EMH, multifocal, moderate.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.
6. Epididymis, head, body: Aspermia, diffuse, severe with minimal tubular dilatation.
7. Epididymis, body: Cribiform change, minimal.
8. Epididymis, tail: Cellular debris and rare spermatid fragments.
9. Kidney, right and left; small intestine; thymus: No significant findings.

Gross necropsy findings: Small testes. Dark red liver. Dark kidney. Pale small intestine with bright yellow fluid.

12-0256

1. Kidney, left: Basophilic tubules, multifocal, minimal.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Micro- and macrovacuolation, diffuse, moderate.
5. Liver: Infiltrates, mononuclear, multifocal and random, mild.
6. Small intestine, enterocytes: Microvacuolation, diffuse, severe.
7. Epididymis, head, body, tail: Aspermia, diffuse, severe with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Epididymis, tail: Cellular debris, mild.
10. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe with mild eosinophilic amorphous interstitial material (fluid).
11. Kidney, right; thymus: No significant findings.

Gross necropsy findings: Small testes. Dark liver. Dark kidneys. Pale small intestine with bright yellow fluid.

12-0297

1. Kidney, right: Basophilic tubules, focal, minimal.
2. Kidney, left: Infiltrates, mononuclear, focal, moderate with basophilic tubules.
3. Spleen: EMH, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Liver: Micro- and macrovacuolation, diffuse, moderate.
6. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
7. Epididymis, head, body: Aspermia, diffuse, severe with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Epididymis, tail: Cellular debris, moderate with rare mature spermatids.
10. Small intestine, enterocytes: Microvacuolation, diffuse, severe.
11. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.
12. Thymus: No significant findings.

Gross necropsy findings: Small testes. Mildly dark liver. Enlarged, dark spleen. Pale intestines with bright yellow fluid content.

12-0314

1. Kidney, right and left; thymus: No significant findings.
2. Liver: Microvacuolation, diffuse, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Spleen: EMH, multifocal, mild.
5. Spleen: Hemosiderosis, multifocal, moderate.
6. Small intestine, enterocytes: Microvacuolation, diffuse, moderate.
7. Epididymis, head, body: Hypospermia, multifocal, moderate with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Epididymis, tail: Cellular debris, moderate with spermatids.
10. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.

Gross necropsy findings: Small testes. Dark liver. Pale-red spleen. Pale small intestine with bright yellow material.

12-0322

1. Kidney, left: Infiltrates, mononuclear, interstitial, focal, minimal.
2. Liver: Microvacuolation, diffuse, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, moderate.
5. Spleen: EMH, multifocal, minimal.
6. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe with eosinophilic amorphous interstitial material (fluid)
7. Epididymis, head, body, tail: Aspermia, diffuse, severe with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Epididymis, tail: Cellular debris, minimal.
10. Kidney, right; thymus: No significant findings.

Gross necropsy findings: Small testes.

12-0341

1. Kidney, left: Basophilic tubules, multifocal, mild with minimal mononuclear infiltrates.
2. Small intestine, enterocytes: Microvacuolation, diffuse, moderate.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Spleen: EMH, multifocal, minimal.
5. Liver: Micro- and macrovacuolation, diffuse, mild.
6. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
7. Epididymis, head, body: Hypospermia, multifocal, moderate with minimal tubular dilatation.
8. Epididymis, body: Cribiform change, mild.
9. Epididymis, tail: Cellular debris, moderate with spermatids.
10. Testes, right and left: Degeneration/atrophy, tubular, diffuse, severe.
11. Kidney, right; thymus: No significant findings.

Gross necropsy findings: Dark liver. Small testes. Dark kidneys. Pale small intestine with bright yellow fluid.

125mg/kg

12-0187

1. Kidney, left: Infiltrates, mononuclear, pelvic, focal, minimal.
2. Liver: Micro- and macrovacuolation, diffuse, moderate.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.
6. Right kidney; thymus; right epididymis; right testis: No significant findings.

Gross necropsy findings: Small amount of bedding n stomach. Bright yellow material in small intestine. Pale small intestine.

12-0204

1. Kidney, right: Basophilic tubules, focal, minimal.
2. Kidney, left: Fibrosis, focal, mild with basophilic tubules and mononuclear infiltrates.
3. Liver: Micro- and macrovacuolation, diffuse, moderate.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
6. Spleen: Hemosiderosis, multifocal, minimal.
7. Spleen: EMH, multifocal, mild.
8. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Mildly dark spleen. Mottled, red liver. Pale small intestine with bright yellow fluid.

12-0223

1. Kidney, right and left: Infiltrates, mononuclear, multifocal, minimal with rare basophilic tubules.
2. Liver: Microvacuolation, multifocal, minimal.
3. Liver: Infiltrates, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.
6. Epididymis, right: Infiltrates, interstitial, focal, minimal.
7. Thymus; right testis: No significant findings.

Gross necropsy findings: Pale small intestine with bright yellow fluid.

12-0224

1. Kidney, right: Basophilic tubules, focal, minimal.
2. Liver: Microvacuolation, multifocal, minimal.
3. Liver: Infiltrates, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild
6. Kidney, left; thymus; testes; epididymis: No significant findings.
7. Testis: Exfoliation, germ cell, multifocal, mild.
8. Epididymis: Infiltrates, lymphocytic, interstitial, multifocal, minimal.

Gross necropsy findings: Pale intestine with bright yellow fluid. Bedding in stomach.

12-0253

1. Kidney, left: Tubular cystic dilatation, focal, mild with minimal mononuclear infiltrates.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: EMH, multifocal, mild.
4. Liver: Infiltrates, multifocal and random, mild.
5. Liver: EMH, focal, minimal.
6. Epididymis, interstitium: Infiltrates, mononuclear, multifocal, mild.
7. Kidney, right; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Dark, red liver. Pale small intestine with pale yellow fluid. Dark kidneys.

12-0275

1. Kidney, left: Infiltrates, mononuclear, focally extensive, mild with basophilic tubules and fibrosis.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Spleen: EMH, multifocal, mild.
4. Liver: Microvacuolation, multifocal, minimal.
5. Liver: Infiltrates, multifocal and random, minimal.
6. Kidney, right; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Dark red liver. Pale small intestine with pale yellow fluid.

12-0293

1. Kidney, left: Infiltrates, mononuclear, multifocal, mild with basophilic tubules.
2. Kidney, right: Basophilic tubules, focal, minimal.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Spleen: EMH, multifocal, mild.
5. Liver: Microvacuolation, multifocal, minimal.
6. Liver: Infiltrates, multifocal and random, mild.
7. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Mildly dark kidneys.

12-0296

1. Kidney, left: Basophilic tubules, focally extensive, moderate with mononuclear infiltrates, fibrosis and cystic tubular dilatation.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Spleen: EMH, multifocal, minimal.
4. Liver: Microvacuolation, multifocal, minimal.
5. Liver: Infiltrates, multifocal and random, mild.

6. Epididymis, tail: Infiltrates, mononuclear, interstitial, focal, mild.
7. Kidney, right; thymus; testis: No significant findings.

Gross necropsy findings: Pale small intestine. Bright yellow fluid in small intestine.

12-0345

1. Kidney, right and left: Basophilic tubules, multifocal, minimal.
2. Liver: Micro- and macrovacuolation, multifocal, minimal.
3. Liver: Infiltrates, multifocal and random, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, mild.
6. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Dark liver. Slightly small spleen. Dark kidneys.

12-0354

1. Kidney, right and left: Basophilic tubules, multifocal, minimal.
2. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Spleen: EMH, multifocal, mild.
5. Thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Pale small intestine with bright yellow fluid. Bedding in stomach.

31.25mg/kg

12-0164

1. Spleen: Hemosiderosis, multifocal, minimal.
2. Liver: Microvacuolation, multifocal, minimal.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Adipose, peri-epididymal: Microgranuloma, focal, minimal.
5. Kidney, right and left; thymus; right and left testes; epididymis: No significant findings.

Gross necropsy findings: Dark red liver. Thymus: left lobe – 2 pale brown 1mm diameter areas.

12-0165

1. Kidney, right and left: Infiltrates, mononuclear, multifocal, minimal.
2. Liver: Micro- and macrovacuolation, multifocal, minimal.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: EMH, multifocal, moderate.
6. Thymus; left testis; epididymis: No significant findings.
7. Lymph node, thymic: Draining hemorrhage, diffuse, moderate.

Gross necropsy findings: Mildly pale liver. Yellow material in stomach.

12-0225

1. Kidney, left: Basophilic tubules, multifocal, mild with minimal mononuclear infiltrates.
2. Spleen: EMH, multifocal, minimal.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Microvacuolation, multifocal, minimal.
5. Liver: Infiltrates, multifocal and random, minimal.
6. Kidney, right; left testis; epididymis: No significant findings.

Gross necropsy findings: Mildly pale liver. Pale small intestine with bright yellow material.

12-0234

1. Kidney, left: Infiltrates, mononuclear, multifocal, mild with basophilic tubules.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Spleen: EMH, multifocal, mild.
4. Liver: Infiltrates, mononuclear, multifocal and random, mild.
5. Liver: Microvacuolation, diffuse, moderate.
6. Kidney, right; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Moderate bedding in stomach. Pale small intestine with bright yellow fluid.

12-0243

1. Kidney, left: Infiltrates, mononuclear, multifocal, minimal with basophilic tubules.
2. Liver: Infiltrates, mononuclear, multifocal and random, mild.
3. Liver: Microvacuolation, diffuse, mild.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Spleen: EMH, multifocal, minimal.
6. Kidney, right; thymus; testis; epididymis: No significant findings.

Gross necropsy findings: Bright yellow material in small intestine.

12-0245

1. Kidney, right: Pelvic dilatation, diffuse, mild.
2. Kidney, left: Infiltrates, mononuclear, multifocal , mild with basophilic tubules and fibrosis.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Spleen: EMH, multifocal, mild.
5. Liver: Infiltrates, mononuclear, multifocal and random, moderate.
6. Liver: Microvacuolation, multifocal, minimal.
7. Thymus; epididymis; testis: No significant findings.

Gross necropsy findings: Dark, red liver. Pale small intestine with bright yellow fluid. Dark kidneys.

12-0255

1. Spleen: EMH, multifocal, minimal.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Liver: Microvacuolation, diffuse, mild.
5. Kidney, right and left; thymus; testis; epididymis: No significant findngs.

Gross necropsy findings: Dark kidneys. Mildly dark spleen. Pale small intestines with bright yellow fluid.

12-0262

1. Spleen: Hemosiderosis, multifocal, mild.
2. Spleen: EMH, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Liver: Microvacuolation, diffuse, mild.
5. Kidney, right and left; testis; epididymis: No significant findings.

Gross necropsy findings: Mildly dark liver. Bedding in stomach.

12-0324

1. Kidney, left: Cystic tubular dilatation, multifocal, mild.
2. Kidney, left: Mineral, focal, minimal.
3. Spleen: EMH, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Liver: Micro- and macrovacuolation, diffuse, moderate.
6. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
7. Liver: EMH, focal, minimal.
8. Testis, left: Residual bodies, atypical, multifocal, mild. (stage XIII)
9. Kidney, right; thymus; epididymis: No significant findings.

Gross necropsy findings: Dark liver. Dark spleen.

12-0351 (died)

1. Kidney, right and left: Autolysis, multifocal, moderate.
2. Liver: Macrovacuolation, diffuse, moderate.
3. Liver; thymus: Congestion, diffuse, severe.
4. Spleen; thymus; testes; epididymis: Autolysis, multifocal, mild.

Gross necropsy findings: Animal found dead. Partial tissues saved.

RECOVERY

Control

12-0174

1. Epididymis: Infiltrates, mononuclear, interstitial, multifocal, minimal.
2. Testis, left: No significant findings.

Gross necropsy findings: Mildly enlarged submandibular lymph nodes. Mildly dark liver.

12-0179

1. Testis, left; epididymis: No significant findings.

Gross necropsy findings: Mildly dark liver. Mildly dark spleen.

12-0194

1. Testis, left; epididymis: No significant findings.

Gross necropsy findings: Dark spleen. Dark kidneys. Hydronephrosis, right kidney.

12-0208

1. Testis, left; epididymis: No significant findings.

Gross necropsy findings: Dark liver

12-0272

1. Testis, left; epididymis: No significant findings.
2. Adipose, epididymis: Infiltrates, mononuclear, multifocal, minimal.

Gross necropsy findings: No gross lesions noted.

12-0294

1. Testis, left; Atrophy, tubular, focal, minimal. (1 tubule)
2. Epididymis: No significant findings.

Gross necropsy findings: Dark liver. Dark spleen.

12-0304
1. Epididymis, body: Cribiform change, diffuse, moderate.
2. Epididymis, head and body: Hypospermia, multifocal, mild.
3. Testis, left: Infiltrates, lymphocytic, perivascular, focal, minimal.

Gross necropsy findings: Dark liver

12-0323
1. Testis, left: Atrophy, tubular, focal. (one tubule)
2. Epididymis, head: Hypospermia, multifocal, minimal.

Gross necropsy findings: No gross lesions noted

12-0332
1. Testis, left: Atrophy, tubular, focal. (2 tubules)
2. Epididymis: No significant findings.

Gross necropsy findings: Dark liver.

12-0365
1. Testis, left: No significant findings.
2. Epididymis, head: Infiltrates, mononuclear, interstitial, multifocal, minimal.

Gross necropsy findings:

500 mg/kg

12-0176
1. Testes, right and left: Degeneration/atrophy, tubular, multifocal, moderate with rare macrovacuolation.
2. Epididymis, head and body: Hypospermia, multifocal, moderate with mild sloughed germ cells and rare interstitial mononuclear infiltrates.
3. Epididymis, tail: Mature spermatids and cellular debris, moderate.
4. Epididymis, body: Cribiform change, diffuse, moderate.

Gross necropsy findings: Small testes. Mildly enlarged dark spleen.

12-0186
1. Testis, left: Degeneration/atrophy, tubular, multifocal, moderate.
2. Epididymis, head and body: Hypospermia, multifocal, moderate with mild sloughed germ cells.
3. Epididymis, tail: Mature spermatids and cellular debris, moderate.
4. Epididymis, body: Cribiform change, diffuse, moderate.

Gross necropsy findings: Dark liver.

12-0215
1. Testis, left: Degeneration/atrophy, tubular, multifocal, severe with multinucleated giant cells.
2. Testis, right: Degeneration, tubular, multifocal, mild with rare macrovacuolation.
3. Epididymis, head: Cellular debris, diffuse, moderate.
4. Epdidymis,left, body and tail: Cellular debris, minimal.
5. Epididymis,left, head, body and tail: Aspermia, diffuse, severe.

6. Epididymis, left, body: Cribiform change, diffuse, mild.

Gross necropsy findings: Small testes. Dark, enlarged spleen. Dark kidneys.

12-0244

1. Testes,right and left : Degeneration/atrophy, tubular, diffuse, mild with rare macrovacuolation.
2. Epididymis, left, head and body: Hypospermia, multifocal, moderate with minimal cellular debris.
3. Epididymis,left, tail: Mature spermatids , diffuse, moderate with minimal cellular debris.
4. Epididymis, left, body: Cribiform change, diffuse, mild.

Gross necropsy findings: Small testes.

12-0283

1. Testes, right and left: Degeneration/atrophy, tubular, diffuse, mild.
2. Epdidiymis, left, tail: Mature spermatids, diffuse, moderate.
3. Epididymis, left, body: Hypospermia, multifocal, moderate.

Gross necropsy findings: Small testes. Dark liver. Moderate amounts of bedding in gastrointestinal system.

12-0295

1. Testis, left: Degeneration, tubular, diffuse, moderate with severe tubular dilatation.
2. Testis, right: Degeneration, tubular, diffuse, moderate.
3. Epididymis, tail: Mature spermatids, moderate.

Note: Lumen of head and body of epididymis was not evaluated; cut did not allow for lumen evaluation. Therefore, this epididymis was excluded from incidence table.

Gross necropsy findings: Small testis, right.

12-0302

1. Testis, left: Degeneration/atrophy, tubular, diffuse, moderate with tubular dilatation and few multinucleated giant cells.
2. Epididymis, left, body: Cribiform change, diffuse, moderate.
3. Epididymis, left, head, body: Hypospermia, multifocal, moderate.
4. Epididymis, left, tail: Mature spermatids, moderate.

Gross necropsy findings: Dark liver. Mildly dark, mottled kidneys.

12-0310

1. Testis, left: Degeneration, tubular, diffuse, mild.
2. Testis, right: Degeneration, tubular diffuse, moderate with dilatation and Sertoli cell vacuolation.
3. Epididymis, left: Aspermia, diffuse, severe with minimal cellular debris.

Gross necropsy findings: Small testes. Dark liver. Dark, mottled kidneys.

12-0333

1. Testis, left and right: Degeneration/atrophy, diffuse, mild.
2. Epididymis, left, head and body: Hypospermia, multifocal, moderate with some cellular debris.
3. Epididymis, left, body: Cribiform change, diffuse, moderate.
4. Epididymis, left, tail: Mature sperm, moderate with cellular debris.

Gross necropsy findings: Small testes. Dark liver. Right kidney, hydronephrosis.

12-0363

1. Testis, left and right: Degeneration/atrophy, diffuse, severe.
2. Epididymis, left, head, body: Hypospermia, multifocal, moderate, with minimal cellular debris.
3. Epididymis, left, body: Cribiform change, diffuse, mild.
4. Epididymis, left, tail: Mature sperm, moderate with cellular debris.

Gross necropsy findings: Small testes. Dark liver.

FEMALES

Control

12-0211

1. Kidney, left: Infiltrates, mononuclear, focal, minimal.
2. Spleen: EMH, multifocal, moderate.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Liver: Microvacuolation, diffuse, minimal.
6. Kidney, right; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Dark liver. Mildly dark kidneys. Mildly dark spleen.

12-0220

1. Spleen: EMH, multifocal, moderate.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Liver: EMH, multifocal, minimal.
5. Liver: Macrovacuolation, portal and midzonal, multifocal, mild.
6. Thymus: Minimal cortical lymphocyte apoptosis and tingible body macrophages, mildly increased medullary lymphocytes, focal loss of corticomedullary junction.
6. Kidney, right and left; small intestine; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Bedding in stomach. Yellow fluid in intestines.

12-0222

1. Spleen: EMH, multifocal, moderate.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Liver: Microvacuolation, multifocal, minimal.
4. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Mildly dark liver.

12-0260

1. Kidney, left: Basophilic tubules, multifocal, mild with mononuclear infiltrates.
2. Liver: Macrovacuolation and glycogen change, portal and midzonal, diffuse, moderate.
3. Spleen: EMH, multifocal, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Thymus: Decreased cortical lymphocytes, increased medullary lymphocytes, no discernible cortico-medullary junction.
6. Kidney, right; uterus; ovary; thymus: No significant findings.

Gross necropsy findings: Pale liver.

12-0289

1. Liver: Infiltrates, mononuclear, multifocal and random, mild with individual hepatocellular necrosis.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Lung: Extravascated red blood cells, multifocal, mild.
5. Thymus: Minimally decreased medullary lymphocytes.
6. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Multiple red spots, lower lobe of lung. Bedding in stomach.

12-0298* -not pregnant

1. Kidney, left: Fibrosis, focal mild with mononuclear infiltrates, tubular loss and cystic tubules.
2. Spleen: EMH, multifocal, minimal.
3. Spleen: Hemosiderosis, multifocal, moderate.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Kidney, right; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Pale small intestines. Not pregnant.

12-0306

1. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
2. Spleen: EMH, multifocal, minimal.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Thymus: Decreased cortical lymphocytes, increased medullary lymphocytes, no discernible cortico-medullary junction.
5. Kidney, right and left; thymus; small intestine; uterus; ovary: No significant findings.

Gross necropsy findings: Dark kidneys. Pale small intestine

12-0309

1. Kidney, left: Mineral, focal, minimal.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: Necrosis, coagulative, mutifocal, mild with mononuclear infiltrates.
5. Liver: Infiltrates, mononuclear, multifocal and random, mild.
6. Liver: Microvacuolation, multifocal, mild.
7. Kidney, right; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Bedding in stomach.

12-0317*- not pregnant

1. Spleen: Hemosiderosis, multifocal, moderate.
2. Small intestine, enterocytes: Microvacuolation, diffuse, severe.
3. Kidney, right and left; liver; thymus; stomach: No significant findings.

Gross necropsy findings: Stomach: 9mmx4, "diverticulum" on serosal side of greater curvature. No mucosal defect noted. Musculature appears to be distending from defect.

12-0339

1. Kidney, right and left, cortex: Epithelial vacuolation, diffuse, severe.
2. Kidney, right and left, cortex: Basophilic tubules, multifocal, moderate with intra-tubular cellular debris.
3. Spleen: EMH, multifocal, moderate.
4. Spleen: Hemosiderosis, multifocal, minimal.
5. Thymus, cortex: Mildly increased cortical lymphocytic apoptosis with tangible body macrophages, increased medullary lymphocytes with no discernible corticomedullary junction.
6. Liver: Vacuolation, multifocal and random, mild with glycogen accumulation.
7. Uterus, ovary: No significant findings.

Gross necropsy findings: Bright yellow fluid in intestines. Bedding in cecum. Fluid filled cysts, right and left ovaries.

31.25mg/kg

12-0168

1. Spleen: EMH, multifocal, moderate.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Liver: Microvacuolation, multifocal, minimal.
5. Small intestine, enterocytes: Microvacuolation, multifocal, mild.
6. Lymph node, mesenteric: Lipid laden macrophages, sinus, mild.
7. Kidney, left and right; thymus: No significant findings.

Gross necropsy findings: Small amount of bedding in stomach. Pale small intestines with bright yellow fluid, yellow tinged mesenteric lymph nodes.

12-0170

1. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
2. Liver: EMH, focal, minimal.
3. Liver: Microvacuolation, diffuse, mild.
4. Spleen: EMH, multifocal, moderate.
5. Spleen: Hemosiderosis, multifocal, minimal.
6. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Pale small intestines.

12-0201

1. Kidney, left, cortex: Basophilic tubules, focal, minimal with mononuclear infiltrates.
2. Kidney, left, pelvis: Dilatation, diffuse, moderate with medullary tubular atrophy/loss.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Liver: Microvacuolation, diffuse, moderate.
5. Spleen: EMH, multifocal, moderate.
6. Spleen: Hemosiderosis, multifocal, moderate.
7. Kidney, right; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: 5mmx2mm mass in fat near ovary. Bedding in stomach. Yellow fluid in intestines.

12-0221

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Liver: Microvacuolation, portal and midzonal, multifocal, mild.

3. Spleen: EMH, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: Congestion, diffuse, moderate.
6. Thymus: Increased medullary lymphocytes, decreased cortical lymphocytes, no discernible corticomedullary junction.
7. Ovary: Hemorrhagic cyst, focal.
8. Kidney, right and left; uterus; stomach: No significant findings.

Gross necropsy findings: Yellow fluid in stomach and intestines.

12-0240

1. Spleen: EMH, multifocal, moderate.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Liver: Infiltrates, multifocal and random, minimal.
4. Kidney, right and left; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Dark liver. Pale small intestine with small amount of bright yellow fluid.

12-0299*-not pregnant

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Spleen: Hemosiderosis, multifocal, moderate.
3. Spleen: EMH, multifocal, minimal.
4. Kidney, right and left; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Dark liver. Pale small intestine with small amount of bright yellow fluid.

12-0300

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Liver: EMH, focal, minimal.
3. Spleen: EMH, multifocal, mild.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Spleen: Congestion, diffuse, moderate.

Kidney, right and left; thymus; uterus ovary: No significant findings.

Gross necropsy findings: Bright yellow fluid in intestines.

12-0346

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Spleen: EMH, multifocal, moderate.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Salivary gland: Acinar dilatation and degeneration, focally extensive, severe.
5. Ovary, corpus luteum: Cyst, hemorrhagic, focal.
5. Kidney, right and left; small intestine; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Mildly enlarged submandibular lymph nodes. Pale small intestines. Bedding in stomach and intestines.

12-0367

1. Kidney, right, medulla: Cyst, focal.
2. Liver: Infiltrates, mononuclear, multifocal, minimal.
3. Thymus: Decreased medullary lymphocytes, indiscernible cortico-medullary junction.
4. Spleen: EMH, multifocal, minimal.

5. Spleen: Hemosiderosis, multifocal, mild.
6. Spleen: Congestion, diffuse, moderate.
7. Kidney, left; ovary; uterus: No significant findings.

Gross necropsy findings: Pales small intestine with bright yellow fluid. Bedding in stomach and intestines.

12-0369

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, moderate.
4. Thymus: Increased medullary lymphocytes.
5. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: No gross lesions noted.

125mg/kg

12-0169

1. Kidney, right and left, cortical epithelial cells: Macro and microvacuoles, multifocal, mild.
2. Spleen: EMH, diffuse, severe.
3. Spleen: Hemosiderosis, multifocal, minimal.
4. Liver: EMH, multifocal, mild.
5. Liver: Infiltrates, mononuclear, focal, minimal.
6. Thymus : Increased meduallry lymphocytes, decreased cortical lymphocytes, no discernible corticomedullary junction.

Gross necropsy findings: Small amount of bedding in stomach; yellow fluid in small intestines. Small thymus.

12-0210

1. Spleen: EMH, diffuse, severe.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Liver: EMH, multifocal, minimal.
5. Lung: Extravascated red blood cells, multifocal, mild with congestion.
6. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Mildly dark liver. Mildly red mottled lungs. Bright yellow fluid in intestines.

12-0212

1. Spleen: EMH, multifocal, moderate.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: Decreased lymphocytes, marginal zone, diffuse, mild.
4. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
5. Liver: EMH, multifocal, minimal.
6. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Bright yellow fluid in intestine. Bedding in stomach

12-0227

1. Kidney, left: Infiltrates, mononuclear, focal, interstitial, mild with basophilic tubules.
2. Liver: Macrovacuoles, multifocal, random, minimal.

3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Spleen: EMH, multifocal, minimal.
5. Spleen: Hemosiderosis, multifocal, minimal.
6. Thymus: Epithelial hyperplasia, cystic, focally extensive.
7. Thymus: Decreased cortical lymphocytes, increased medullary lymphocytes.
8. Kidney, right; uterus; ovary: No significant findings.

Gross necropsy findings: Pale intestines. Bedding in stomach and intestines.

12-0257

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: Congestion, diffuse, mild.
4. Liver: Infiltrates, mononuclear, multifocal and random, mild.
5. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Yellow fluid and bedding in intestines. Bedding in stomach.

12-0259

1. Kidney, right and left, cortex: Micro- and macrovacuolation, diffuse, marked with moderate basophilic tubules and few intratubular necrotic debris.
2. Liver: Glycogen accumulation, portal and midzonal, diffuse, moderate.
3. Liver: Apoptosis, hepatocellular, centrilobular, multifocal, minimal.
4. Thymus: Atrophy, diffuse, severe.
5. Spleen: EMH, multifocal, moderate.
6. Spleen: Hemosiderosis, multifocal, minimal.
7. Spleen: Hyperplasia, lymphoid, marginal zone, multifocal, mild.
8. Ovary; uterus; lung: No significant findings.

Gross necropsy findings: Yellow tinged subcutaneous brown fat. Pale brown liver. Mottled red lungs. Mildly dark kidneys. Overall body condition thin. Minimal visceral fat.

12-0261

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Spleen: EMH, multifocal, mild.
3. Spleen: Hemosiderosis, multifocal, mild.
4. Kidney, right and left; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Mildly distended stomach with bedding. Mild amount of bedding in small intestines. Pale small intestines.

12-0278

1. Liver: Microvacuolation, multifocal, moderate.
2. Spleen: EMH, multifocal, moderate.
3. Spleen: Hemosiderosis, multifocal, moderate.
4. Salivary gland: Acinar dilatation and degeneration, focally extensive, severe.
5. Thymus: Increased meduallary lymphocytes.
5. Kidney, right and left; ovary; uterus: No significant findings.

Gross necropsy findings: Mildly enlarged submandibular lymph nodes. Mildly dark liver. Pale intestines. Small amount of bedding in stomach.

12-0291

1. Kidney, left: Infiltrates, mononuclear, focal, minimal.
2. Kidney, left, cortical cells: Microvacuolation, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Thymus: Decreased cortical lymphocytes and increased medullary lymphocytes.
5. Spleen: EMH, multifocal, minimal.
6. Spleen: Hemosiderosis, multifocal, minimal.
7. Spleen: Congestion, diffuse, moderate.
8. Kidney, right; ovary; uterus: No significant findings.

Gross necropsy findings: Pale small intestine. Moderate amount of bedding in gastrointestinal system.

12-0327

1. Kidney, right: Pelvic dilatation, diffuse, severe with severe cortical and medullary atrophy/loss.
2. Kidney, left: Pelvic dilatation, diffuse, moderate with moderate medullary atrophy/loss.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Liver: Congestion, multifocal, moderate.
5. Liver: EMH, focal, minimal.
6. Spleen: EMH, multifocal, moderate.
7. Spleen: Hemosiderosis, multifocal, mild.
8. Thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Dark liver. Right kidney enlarged and cystic. Left kidney cystic.

500mg/kg

12-0166

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Kidney, right and left; liver; uterus; ovary; thymus: No significant findings.

Gross necropsy findings: Mildly dark liver and spleen.

12-0167

1. Kidney, left: Infiltrates, mononuclear, focal, minimal.
2. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
3. Spleen: EMH, multifocal, moderate.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Kidney, right; small intestine; thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Pale intestines, yellow fluid in stomach.

12-0171*- not pregnant

1. Liver: Infiltrates, mononuclear, multifocal and random, mild.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Kidney, right and left; small intestine; uterus; ovary: No significant findings.
4. Thymus: Not examined.

Gross necropsy findings: Not pregnant; yellow fluid in intestines.

12-0198

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Kidney, right and left; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Bedding and yellow fluid in intestines.

12-0229*-not pregnant

1. Spleen: EMH, multifocal, minimal.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Uterus, lumen: Dilatation, diffuse, severe.
4. Kidney, right and left; thymus; liver; ovary: No significant findings.

Note: Uterus is in proestrus.

Gross necropsy findings: Fluid filled uterus, mild.

12-0258

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Liver: Macrovacuolation, portal and midzonal, multifocal, moderate.
4. Liver: EMH, multifocal, minimal.
5. Uterus: Dilatation, diffuse, severe.
6. Kidney, right and left; ovary: No significant findings.
7. Thymus: Not examined.

Note: Uterus is in proestrus.

Gross necropsy findings: Dilated uterus. Mildly dark kidneys. Bright yellow fluid in small intestines.

12-0279

1. Liver: Infiltrates, mononuclear, focal, minimal.
2. Thymus: Increased medullary lymphocytes, decreased cortical lymphocytes, increased apoptotic bodies and tingible body macrophages, no discernible corticomedullary junction.
3. Spleen: EMH, multifocal, minimal.
4. Spleen: Hemosiderosis, multifocal, mild.
5. Kidney, right and left; cecum; uterus; ovary: No significant findings.

Gross necropsy findings: Pale yellow staining fur on abdomen and vulva. Mildly gas distended cecum.

12-0308

1. Spleen: EMH, multifocal, minimal.
2. Spleen: Hemosiderosis, multifocal, mild.
3. Liver: Infiltrates, mononuclear, multifocal and random, minimal.
4. Kidney, right and left; thymus; ovary; uterus: No significant findings.

Gross necropsy findings: Bedding and yellow fluid in intestines.

12-0336

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Spleen: Decreased lymphocytes, mantle zone.
4. Kidney, right and left; liver; thymus; uterus; ovary: No significant findings.
5. Thymus: Decreased medullary lymphocytes.

Gross necropsy findings: Small amount of fluid in small intestine.

12-0348

1. Spleen: EMH, multifocal, mild.
2. Spleen: Hemosiderosis, multifocal, minimal.
3. Liver: Infiltrates, mononuclear, multifocal and random, mild.
4. Thymus; uterus; ovary: No significant findings.

Gross necropsy findings: Mildly distended stomach with bedding. Mild amount of bedding in small intestines. Pale small intestines.

8. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The portion of the study described in this contributing scientist report, including the tissue processing piece conducted at the United States Army Medical Research Institute of Chemical Defense's Comparative Pathology Branch, was conducted in compliance with Title 40, Code of Federal Regulations (CFR) Part 792, Good Laboratory Practice Standards.

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11 JULY 2013
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Appendix P

Statistical Analysis Report

Statistical Analysis on USAPHC Toxicology Protocol Number: 0FP4-93-12-03-03

Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

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I. Study Background

The primary objective of this study was to determine the initial reproductive and developmental toxicity of 3-nitro-1,2,4-triazol-5-one (NTO) through the use of a screening test. The secondary objective was to confirm the effects of repeated-dose exposure to NTO using different exposure durations and dose levels than previously evaluated.

Adult male and female rates were singly housed and exposed to NTO for 2 weeks, then pair-housed for mating and dosed for another 2 weeks. Males were euthanized after pair-housing while females continued dosing until 3-4 days post-partum and were then euthanized.

A separate group of males was used as a satellite/recovery group for the control and highest dose of NTO. These animals were dosed concurrently with the males in the reproductive study but not euthanized until 14-28 days after dosing was completed.

Body weight, food consumption, relevant organ weights and blood analysis of the adults dosed with NTO were compared with each other and with non-NTO controls to determine effects. In addition, analyses of reproductive outcomes and litter characteristics were also performed.

Each dose group began with 10 animals. One male died in the study so the base sample size for the 31.25 mg/kg dose group was reduced to 9 animals. For the females, five did not get pregnant and therefore the baseline sample size for pregnancy, post-partum, and necropsy measures was reduced. Differences from these bases, due to sporadic missing data, are noted in specific tables throughout the report.

Table 1. Number of Animals per Treatment Group

Treatment	# Mated Male Rats	# Satellite/ Recovery Male Rats	# Female Rats	
			For Pre-Pregnancy measures	For Gestation, Post-Partum, and Necropsy measures
Control	10	10	10	8
NTO 31.25 mg/kg	9	--	10	9
NTO 125 mg/kg	10	--	10	10
NTO 500 mg/kg	10	10	10	8

II. Summary of Results

There were several statistically significant results in the study; however, given the large number of statistical tests performed, most the 'significance' was probably random. Only the effect of NTO on the testes, epididymides, and sperm count was consistent for the males. For the females, some consistent reduction in spleen and uterus weights was shown.

Table 2. Summary of Statistically Significant Results

Category	Males	Satellite/Recovery Males	Females
Clinical Chemistry (17 measures)	TBIL 500>125	--	PHOS 500>125
Hematology (20 measures)	MCV 500>31.25 MCH 500>31.25 PLT 500<125	--	%BASO 500>125
Body Weight* - Absolute weight - Weekly gain	--	--	Weekly gain - Pre-Pregnancy 500<31.25
Food Consumption*	--	--	Pre-Pregnancy 500<31.25

Organ Weight (9 organs, 3 measures each**)
 	
- Absolute weight
- Organ to body wt
- Organ to brain wt
	Testes (all measures)
	• 500<control
	• 500<125
	• 500<31.25
	
	Epididymides (all measures)
	• 500<control
	• 500<31.25
	• 500<125
		Testes (all measures)
		• 500<control
		
		Epididymides (all measures)
		• 500<control
			Brain
			• Absolute 31.25<control
			
			Spleen
			• Absolute
			  • 31.25<control
			  • 500<control
			• Organ to body ratio
			  • 31.25<control
			  • 500<control
			
			Uterus without Outlier
			• Absolute 500<125
			• Organ to brain ratio 500<125

Sperm Count	500<control 500<31.25 500<125	500<control	NA
Prothrombin Time	--	--	--
Reproductive Data (4 measures)	NA	NA	Sample sizes too small to tell
Litter Measures (12 measures)	--	--	--

* Female body weight and food consumption broken into pre-pregnancy, gestation, and post-partum.
** There could be no organ to brain weight ratio for the brain itself.

III. Statistical Analysis Methods

Analyses were conducted for males and females separately. SPSS 16.0 was used to perform all analyses and statistical significance was defined as $p \leq .05$.

A. One Time Measurement Variables for Adults

For hematology, clinical chemistry, organ to brain weight ratios, organ to body-weight ratios, sperm count data, and litter/pup parameters, the dose groups were compared using a one-factor analysis of variance (ANOVA). If the dose group effect was significant, a Tukey post hoc test was used to compare pairs of dose groups.

- The Levene's test was used to determine the variance of the groups. The Tukey post hoc test was used because group variances were equal.
- Data was checked for normality by plotting residuals. If data was not normal it was natural log transformed. If transformation still did not satisfy ANOVA assumptions, a non-parametric Kruskal-Wallis (K-W) test was used to analyze dose group differences.

For absolute organ weights, comparison of the dose groups was made using an analysis of covariance (ANCOVA), with body weight at the end of the study as the covariate. Even though the dose groups were assigned at Day 0 to keep the average starting weight for each dose group similar, the weights could have changed during the study dependent on the dose group. The ANCOVA adjusted for any differences in terminal body weights among the dose groups, because heavier animals would tend to have heavier organs. If the dose group effect was significant, a Sidak post hoc test was used to compare pairs of dose groups and dose groups to the control group.

B. Repeated Measure Variables for Adults

Weekly changes in body weight and food consumption were compared using repeated measures ANOVA. If the dose effect in the ANOVA was significant, a Tukey post hoc test was used to compare pairs of dose groups. If the interaction effect of week and dose group was statistically significant, weekly means were compared but overall dose group means were not because results would have been inconclusive. Just like the one-way ANOVAs, verification of normally distributed data (residual plots) and equal variances among dose groups (Levene's test) assumptions was performed.

C. Reproductive Data

The distributions of reproductive characteristics were compared descriptively. The sample sizes for each group were too small to test statistically. To test for differences in the count distribution, a chi-square test would have been appropriate. However, given the sample size and the high proportion of females achieving the various reproductive endpoints, the expected value of many of the cells would have been less than 5 and violated the assumptions of a chi-square test.

IV. Results – Mated Males

A. Blood Chemistry

Statistically significant differences between dose groups were not present for any of the clinical chemistry metrics except for total bilirubin (TBIL) and inorganic phosphorus (PHOS). For TBIL, the 500 mg/kg dose group had a significantly higher value compared to the 125 mg/kg dose group. For PHOS, even though the p-value was less than .05, the post-hoc test found no significant differences between dose groups after adjusting for multiple comparisons.

Table 3. Mated Male Clinical Chemistry Results

		ALB		ALKP		ALT		AST	
Dose Group	**N***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	10	3.42	0.21	209.10	55.25	55.20	6.68	88.30	13.14
31.25 mg/kg	8	3.33	0.13	230.63	54.88	53.50	8.26	80.63	14.97
125 mg/kg	8	3.43	0.15	231.88	46.05	51.88	4.22	82.88	18.14
500 mg/kg	10	3.46	0.23	199.90	52.37	58.00	8.06	104.5	52.01
P-Value		p = .507		p = .491		p = .309		p = .340	

Dose Group	**N***	**CA**		**CHOL**		**CREA**		**GLOB**	
Control	10	11.48	0.42	62.50	14.03	0.52	0.08	3.41	0.24
31.25 mg/kg	8	11.49	0.31	71.75	15.31	0.51	0.11	3.24	0.21
125 mg/kg	8	11.49	0.53	70.13	11.26	0.56	0.09	3.36	0.87
500 mg/kg	10	11.59	0.41	64.80	13.41	0.46	0.07	3.44	0.16
P-Value		p = .928		p = .443		p = .124		p = .795	

Dose Group	**N***	**GLU**		**PHOS**		**TBIL**		**TP**	
Control	10	205.10	58.98	11.44	0.84	0.74	0.28	6.82	0.37
31.25 mg/kg	8	210.63	53.68	11.44	1.08	0.65	0.20	6.59	0.22
125 mg/kg	8	209.50	61.34	12.34	1.12	0.54	0.17	6.78	0.84
500 mg/kg	10	216.30	41.46	12.56	0.88	0.90	0.37	6.90	0.26
P-Value		p = .974		p = .030 no post-hoc difference		Lognormal p = .037 500 mg/kg > 125 mg/kg (p=.023)		p = .030	

Dose Group	**N***	**Cl**		**Na**		**K**	
Control	8	104.25	1.28	151.88	1.81	10.01	0.92
31.25 mg/kg	8	102.63	1.06	150.63	1.6	9.69	1.35
125 mg/kg	6	103.67	1.51	151.83	1.33	9.68	0.45
500 mg/kg	5	104.00	1.22	152.6	1.52	9.58	0.86
P-Value		p = .088		p = .182		p = .858	

Dose Group	N*	LDH		BUN	
Control	9	604.33	342.39	14.11	3.52
31.25 mg/kg	8	576.00	203.23	12.88	2.03
125 mg/kg	8	517.75	109.40	12.88	2.10
500 mg/kg	10	755.60	419.99	13.50	1.90
P-Value		p = .399		p = .693	

* Missing all clinical chemistry data for one 31.25 mg/kg animal and two 125 mg/kg animals. In addition, missing Cl, Na and K data from two control animals, two 125 mg/kg animals, and five 500 mg/kg animals. Also, missing LDH and BUN from one of the two control animals.

B. Hematology

Statistically significant differences between dose groups were not present for any of the hematology metrics except mean cell volume (MCV), mean cell hemoglobin (MCH), and platelets (PLT). For MCV and MCH, the 500 mg/kg dose group had a significantly higher average compared to the 31.25 mg/kg group. For PLT, the 500 mg/kg dose group had a significantly lower average compared to the 125 mg/kg group.

Table 4. Mated Male Hematology Results

Dose Group	N	NEU (K/uL)		NEU (%N)		LYM (K/uL)		LYM (%L)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	10	2.00	1.05	13.34	6.24	10.68	2.13	72.04	10.91
31.25 mg/kg	9	1.43	0.35	10.8	3.43	10.96	3.74	76.86	5.33
125 mg/kg	10	2.12	0.76	14.03	4.04	11.55	4.87	73.3	6.65
500 mg/kg	10	1.62	0.62	12.19	3.93	10.6	4.40	73.28	7.35
P-Value		K-W p = .095		Lognormal p = .304		p = .948		K-W p = .774	

Dose Group	N	MONO (K/uL)		MONO (%M)		EOS (K/uL)		EOS (%E)	
Control	10	0.95	0.60	6.46	4.13	0.11	0.03	0.76	0.22
31.25 mg/kg	9	0.72	0.33	5.12	1.73	0.11	0.04	0.79	0.23
125 mg/kg	10	0.87	0.40	5.67	2.09	0.13	0.06	0.83	0.35
500 mg/kg	10	0.9	0.45	6.21	2.23	0.15	0.09	0.99	0.48
P-Value		Lognormal p = .817		Lognormal p = .738		p = .370		p = .421	

Dose Group	N	BASO (K/uL)		BASO (%B)		RBC (M/uL)		HGB (g/dL)	
Control	10	1.09	0.65	7.43	4.49	8.43	0.32	17.23	1.01
31.25 mg/kg	9	0.92	0.42	6.44	2.04	8.44	0.41	16.64	0.65
125 mg/kg	10	0.95	0.43	6.17	2.68	8.53	0.36	17.32	0.96
500 mg/kg	10	1.05	0.57	7.33	2.92	8.35	0.48	17.59	0.97
P-Value		Lognormal p = .950		Lognormal p = .797		p = .785		p = .171	

Dose Group	N	HCT (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
Control	10	47.44	1.60	56.29	1.48	20.45	0.95	36.33	1.78
31.25 mg/kg	9	46.03	2.00	54.57	1.01	19.72	0.74	36.18	1.02
125 mg/kg	10	47.16	2.00	55.3	1.64	20.33	0.92	36.73	1.21
500 mg/kg	10	47.14	1.71	56.56	1.92	21.10	0.94	37.32	1.47
P-Value		p = .375		p = .032 500 mg/kg > 31.25 mg/kg (p=.041)		p = .019 500 mg/kg > 31.25 mg/kg (p=.010)		p = .298	

Dose Group	N	RDW (%)		PLT (K/uL)		MPV (fL)		WBC (K/uL)	
Control	10	14.91	1.27	680.9	262.17	5.62	0.69	14.82	1.76
31.25 mg/kg	9	15.27	0.92	704.22	226.55	5.47	0.50	14.15	4.21
125 mg/kg	10	15.54	0.59	878.6	248.88	5.38	0.31	15.63	5.76
500 mg/kg	10	15.38	0.76	567.6	216.40	5.68	0.46	14.32	5.40
P-Value		p = .479		p = .050 500 mg/kg < 125 mg/kg (p=.031)		p = .547		p = .893	

C. Body Weight

There were no significant differences among dose groups for their body weights during the twenty-eight day study. Although the control group typically had the heaviest body weight, especially towards the end of the study, this difference was not enough to be statistically significant.

Table 5. Mated Male Total Body Weight

Dose Group	N	Day 0		Day 7		Day 14	
		Mean	SD	Mean	SD	Mean	SD
Control	10	339.60	22.98	397.70	25.55	450.70	29.99
31.25 mg/kg	9	332.67	19.63	387.11	28.02	434.22	33.38
125 mg/kg	10	338.20	19.44	388.90	21.66	430.90	22.17
500 mg/kg	10	344.90	24.69	394.30	29.60	435.00	35.39

Dose Group	N	Day 21		Day 27		Day 28	
Control	10	483.90	31.26	519.80	31.02	502.30	33.01
31.25 mg/kg	9	462.11	37.24	494.78	46.45	477.67	43.54
125 mg/kg	10	459.70	24.98	491.90	28.88	473.90	28.04
500 mg/kg	10	462.40	46.47	498.50	53.13	478.60	52.37
P-value	Repeated measures Dose Effect p = .551						

Body weight change followed the same pattern as absolute body weight; all groups increased each week, with the control group typically having a slightly higher average weekly increase compared to all other dose groups. Again, it was not enough to be statistically significant.

Table 6. Mated Male Body Weight Change									
Dose Group	N	Day 7 - Day 0		Day 14 - Day 7		Day 21 - Day 14		Day 27 - Day 21	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	10	58.10	4.91	53.00	5.72	33.20	6.84	35.90	4.36
31.25 mg/kg	9	54.44	10.04	47.11	6.88	27.89	9.66	32.67	10.26
125 mg/kg	10	50.70	6.00	42.00	7.10	28.80	7.08	32.20	8.20
500 mg/kg	10	49.40	12.99	40.70	14.94	27.40	14.66	36.10	8.14
P-value		Repeated measures Dose Effect p = .100							

D. Food Consumption

Food consumption averages from week to week were similar across all four dose groups. P-values, well above .05, additionally indicated that there were no significant differences between dose groups from day 0 to day 14 and from day 21 to day 27.

Table 7. Mated Male Food Consumption Differences from Day 0 to 27									
Dose Group	N	Day 7 - Day 0		Day 14 - Day 7		Day 21 - Day 14*	Day 27 - Day 21**		
		Mean	SD	Mean	SD		N	Mean	SD
Control	9***	212.03	18.30	213.13	19.08		8	169.79	18.81
31.25 mg/kg	10	209.85	22.74	205.71	21.85		7	170.39	15.92
125 mg/kg	10	205.91	14.05	196.39	16.54		10	163.37	14.46
500 mg/kg	10	212.15	25.31	202.26	26.96		8	164.51	23.25
P-value		Repeated measures Dose Effect p = .775					p = .810		

* Day 21 - Day 14 data was not collected because all animals were pair-housed during that time.

** Food consumption values were excluded from repeated measures due to missing data that was not missing at random. Results should be taken with precaution.

*** ID 12-0207 was excluded from results because rat was crumbling food on cage floor.

E. Organ Weight

Organ weights, organ to body weight ratios, and organ to brain weight ratios were very similar among dose groups except for the two male reproductive organs. P-values for all three organ measures in both testes and epididymides were statistically significant. The 500 mg/kg dose group had significantly lower averages compared to the control group, the 31.25 mg/kg dose group, and 125 mg/kg dose group. The initial ANOVA on the heart to brain weight ratio indicated significance, but the post-hoc test found no differences between dose groups after adjusting for multiple comparisons.

Table 8. Mated Male Organ Weight Results

Dose Group	N	Adrenals Unadjusted Weight		Adrenals to Body Weight Ratio		Adrenals to Brain Weight Ratio	
		Mean	SD	Mean	SD	Mean	SD
Control	10	0.086	0.015	0.00017	0.00003	0.041	0.009
31.25 mg/kg	9	0.083	0.016	0.00018	0.00004	0.039	0.007
125 mg/kg	10	0.085	0.012	0.00018	0.00003	0.038	0.005
500 mg/kg	10	0.091	0.018	0.00019	0.00004	0.043	0.008
P-Value		p = .706		p = .586		p = .483	

Dose Group	N	Brain Unadjusted Weight		Brain to Body Weight Ratio		Brain to Brain Weight Ratio
Control	10	2.13	0.11	0.00425	0.00037	Not applicable
31.25 mg/kg	9	2.16	0.08	0.00454	0.00039	
125 mg/kg	10	2.20	0.05	0.00466	0.00030	
500 mg/kg	10	2.12	0.05	0.00447	0.00050	
P-Value		p = .083		p = .153		

Dose Group	N	Epididymides Unadjusted Weight		Epididymides to Body Weight Ratio		Epididymides to Brain Weight Ratio	
Control	10	1.28	0.19	0.00254	0.00035	0.601	0.082
31.25 mg/kg	9	1.23	0.09	0.00258	0.00017	0.570	0.045
125 mg/kg	10	1.32	0.08	0.00279	0.00025	0.598	0.040
500 mg/kg	10	0.83	0.14	0.00175	0.00034	0.392	0.067
P-Value		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)	

Dose Group	N	Heart Unadjusted Weight		Heart to Body Weight Ratio		Heart to Brain Weight Ratio	
Control	10	1.89	0.16	0.00377	0.00023	0.894	0.106
31.25 mg/kg	9	1.72	0.19	0.00361	0.00025	0.799	0.083
125 mg/kg	10	1.77	0.12	0.00375	0.00021	0.806	0.066
500 mg/kg	10	1.73	0.14	0.00363	0.00032	0.816	0.062
P-Value		p = .160		p = .415		Lognormal p = .048 no post-hoc difference	

Dose Group	N	Kidneys Unadjusted Weight		Kidneys to Body Weight Ratio		Kidneys to Brain Weight Ratio	
Control	10	3.37	0.23	0.00672	0.00033	1.59	0.16
31.25 mg/kg	9	3.36	0.39	0.00703	0.00054	1.56	0.18
125 mg/kg	10	3.16	0.20	0.00668	0.00033	1.44	0.09
500 mg/kg	10	3.28	0.45	0.00686	0.00065	1.55	0.21
P-Value		p = .438		p = .390		p = .182	

Dose Group	N	Liver Unadjusted Weight		Liver to Body Weight Ratio		Liver to Brain Weight Ratio	
Control	10	17.71	1.41	0.03529	0.00219	8.36	0.85
31.25 mg/kg	9	17.24	2.66	0.03599	0.00387	8.00	1.24
125 mg/kg	10	16.47	1.31	0.03473	0.00165	7.48	0.66
500 mg/kg	10	16.60	2.46	0.03464	0.00194	7.85	1.15
P-Value		p = .616		p = .649		p = .277	

Dose Group	N	Spleen Unadjusted Weight		Spleen to Body Weight Ratio		Spleen to Brain Weight Ratio	
Control	10	0.934	0.173	0.00186	0.00033	0.443	0.103
31.25 mg/kg	9	0.853	0.101	0.00179	0.00019	0.395	0.039
125 mg/kg	10	0.863	0.062	0.00183	0.00015	0.393	0.036
500 mg/kg	10	0.925	0.168	0.00193	0.00022	0.436	0.075
P-Value		K-W p = .581		K-W p = .493		K-W p = .407	

Dose Group	N	Testes Unadjusted Weight		Testes to Body Weight Ratio		Testes to Brain Weight Ratio	
Control	10	3.55	0.39	0.00709	0.00080	1.678	0.227
31.25 mg/kg	9	3.63	0.22	0.00764	0.00066	1.686	0.112
125 mg/kg	10	3.76	0.21	0.00798	0.00083	1.710	0.119
500 mg/kg	10	1.48	0.18	0.00310	0.00040	0.696	0.073
P-Value		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)		p = .000 500 mg/kg < Control 500 mg/kg < 31.25 mg/kg 500 mg/kg < 125 mg/kg (p=.000 for all)	

Dose Group	N	Thymus Unadjusted Weight		Thymus to Body Weight Ratio		Thymus to Brain Weight Ratio	
Control	10	0.646	0.080	0.00129	0.00016	0.304	0.041
31.25 mg/kg	9	0.649	0.110	0.00135	0.00013	0.301	0.051
125 mg/kg	10	0.594	0.073	0.00125	0.00014	0.270	0.037
500 mg/kg	10	0.631	0.159	0.00131	0.00025	0.298	0.073
P-Value		p = .804		p = .679		p = .457	

F. Sperm Count

The 500 mg/kg dose group was severely impacted by NTO as indicated by a significantly lower sperm count per sample compared to the other three dose groups. The control, 31.25 mg/kg, and 125 mg/kg dose groups had very similar results to each other.

Table 9. Mated Male Sperm Count/Sample (million/gram)

Dose Group	N	Mean	SD
Control	10	168.0	22.3
31.25 mg/kg	9	153.5	32.8
125 mg/kg	10	146.5	47.6
500 mg/kg	10	11.5	10.2
P-value		p = .000 500 mg/kg < Control (p=.000) 500 mg/kg < 31.25 mg/kg (p=.000) 500 mg/kg < 125 mg/kg (p=.000)	

G. Prothrombin Time

Prothrombin times among the dose groups were similar with the p-value well above .05. The largest difference was between the control and the 125 mg/kg dose groups with a difference of .38 seconds.

Table 10. Mated Male Prothrombin Time (seconds)

Dose Group	N	Mean	SD
Control	10	9.83	0.69
31.25 mg/kg	9	9.70	0.66
125 mg/kg	10	9.45	0.60
500 mg/kg	10	9.68	0.55
P-value		p = .594	

IV. Results – Satellite/Recovery Males

A. Clinical Chemistry

There were no significant differences found between the control group and 500 mg/kg dose group averages for any of the seventeen clinical chemistry measures.

Table 11. Satellite/Recovery Male Clinical Chemistry Results

	ALB		ALKP		ALT		AST		Cl	
Dose Group	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	3.53	0.13	121.90	28.76	41.00	7.62	71.80	14.73	104.40	1.58
500 mg/kg	3.46	0.18	116.90	22.82	41.20	10.33	64.90	17.33	104.00	1.63
P-Value	p = .322		p = .672		p = .961		p = .350		p = .584	

Dose Group	**BUN**		**CA**		**CHOL**		**CREA**	
Control	17.00	2.45	12.04	0.54	96.10	21.54	0.61	0.11
500 mg/kg	18.00	6.36	11.74	0.37	92.20	16.75	0.56	0.10
P-Value	p = .648		p = .166		p = .657		p = .295	

Dose Group	**GLOB**		**GLU**		**LDH**		**PHOS**	
Control	3.24	0.21	253.10	43.04	405.50	98.38	11.08	0.78
500 mg/kg	3.32	0.23	222.80	40.47	373.40	184.34	10.90	0.95
P-Value	p = .424		p = .122		p = .633		p = .647	

Dose Group	**TBIL**		**TP**		**Na**		**K**	
Control	0.47	0.17	6.78	0.23	151.30	1.25	9.18	1.39
500 mg/kg	0.39	0.18	6.75	0.31	150.90	2.02	9.26	0.84
P-Value	p = .320		p = .807		p = .602		p = .878	

N= 10 for Control and 500 mg/kg dose groups within each clinical chemistry measure.

B. Hematology

There were no significant differences found between the control group and 500 mg/kg dose group averages for any of the twenty hematology measures.

Table 12. Satellite/Recovery Male Hematology Results								
Dose Group	NEU (K/uL)		NEU (%N)		LYM (K/uL)		LYM (%L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1.33	0.44	9.37	2.68	11.19	3.56	76.93	5.48
500 mg/kg	1.40	0.37	10.43	3.36	11.13	3.69	77.78	5.48
P-Value	p = .698		p = .446		p = .971		p = .733	
Dose Group	MONO (K/uL)		MONO (%M)		EOS (K/uL)		EOS (%E)	
Control	1.04	0.32	7.41	1.75	0.19	0.08	1.35	0.40
500 mg/kg	0.88	0.36	6.38	2.16	0.18	0.08	1.30	0.50
P-Value	p = .308		p = .258		p = .763		p = .793	
Dose Group	BASO (K/uL)		BASO (%B)		RBC (M/uL)		HGB (g/dL)	
Control	0.69	0.20	4.94	1.36	8.38	0.60	15.77	0.91
500 mg/kg	0.58	0.21	4.10	1.23	8.28	0.55	15.69	0.61
P-Value	p = .257		p = .166		p = .685		p = .820	
Dose Group	HCT (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
Control	43.72	2.50	52.21	1.44	18.84	0.53	36.09	0.40
500 mg/kg	43.62	1.87	52.79	2.24	19.01	0.82	36.00	0.33
P-Value	p = .920		p = .500		p = .589		p = .589	
Dose Group	RDW (%)		PLT (K/uL)		MPV (fL)		WBC (K/uL)	
Control	16.29	0.93	985.70	205.10	5.27	0.43	14.45	4.21
500 mg/kg	16.33	0.89	1011.50	109.67	5.10	0.27	14.18	4.22
P-Value	p = .923		p = .730		p = .307		p = .888	

N= 10 for Control and 500 mg/kg dose groups within each hematology measure.

C. Body Weight

Body weights for the control group were slightly higher by the end of the initial study period and this pattern continued through the recovery period. However, this difference was not enough to be statistically significant.

Table 13. Satellite/Recovery Male Total Body Weight								
Dose Group	**Day 0**		**Day 7**		**Day 14**			
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Control	337.6	28.9	394.4	32.4	445.1	35.0		
500 mg/kg	338.0	30.4	390.7	34.9	437.5	40.3		
Dose Group	**Day 21**		**Day 28**		**Recovery Day 35**			
Control	487.6	40.9	531.0	47.5	555.0	50.3		
500 mg/kg	476.9	43.7	514.6	45.6	538.4	49.3		
Dose Group	**Recovery Day 42**		**Recovery Day 49**		**Recovery Day 55**		**Recovery Day 56**	
Control	577.3	48.7	601.3	52.1	616.3	52.5	597.1	52.3
500 mg/kg	562.5	50.3	585.6	52.9	601.9	54.0	582.0	52.2
Repeated Measures Dose Group Effect p-value = .565								

N = 10 for the control and 500 mg/kg dose groups at all 8 food consumption data points.

Week to week body weight gain was slightly higher in the control group for the initial 28-day study, however, in the recovery period, weight gain was very similar between the two dose groups. Again, this difference was not enough to be statistically significant.

Table 14. Satellite/Recovery Male Body Weight Gain								
Dose Group	**Day 7 - Day 0**		**Day 14 - Day 7**		**Day 21 - Day 14**		**Day 28 - Day 21**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	56.80	8.46	50.70	6.85	42.50	9.89	43.40	10.21
500 mg/kg	52.70	9.10	46.80	6.89	39.40	6.11	37.70	5.17
Dose Group	**Recovery Day 35 - Day 28**		**Recovery Day 42 - Recovery Day 35**		**Recovery Day 49 - Recovery Day 35**		**Recovery Day 55 - Recovery Day 49**	
Control	24.00	7.38	22.30	7.26	24.00	7.18	15.00	4.62
500 mg/kg	23.80	9.35	24.10	9.83	23.10	7.34	16.30	7.38
Repeated Measures Dose Group Effect p-value = .304								

N = 10 for the control and 500 mg/kg dose groups at all 8 food consumption data points.

D. Food Consumption

Total food consumption for the 500mg/kg group was lower than the control group; however that difference was not statistically significant highlighted by the p-value well above .05.

Table 15. Satellite/Recovery Male Food Consumption from Day 0 to Recovery Day 55		
Dose Group	**Mean**	**SD**
Control	1724.97	103.27
500 mg/kg	1661.84	109.68
P-value	p = .202	

N = 10 for the control and 500 mg/kg dose groups

E. Organ Weight

Besides the two male reproductive organs (testes and epididymides), differences between organ weights, organ to body weight ratios, and organ to brain weight ratios were non-significant, all having p-values well above .05. However, for the epididymides and testes, the 500 mg/kg dose group had averages that were significantly lower than the control group on all three organ weight measures.

Table 16. Satellite/Recovery Male Organ Weight Results						
Dose Group	**Adrenals Unadjusted Weight**		**Adrenals to Body Weight Ratio**		**Adrenals to Brain Weight Ratio**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	0.07	0.01	0.00011	0.00002	0.030	0.004
500 mg/kg	0.07	0.01	0.00012	0.00002	0.030	0.005
P-Value	p = .790		p = .598		p = .866	
Dose Group	**Brain Unadjusted Weight**		**Brain to Body Weight Ratio**		**Brain to Brain Weight Ratio**	
Control	2.24	0.08	0.00378	0.00032	Not applicable	
500 mg/kg	2.24	0.12	0.00386	0.00022		
P-Value	p = .940		p = .510			
Dose Group	**Epididymides Unadjusted Weight**		**Epididymides to Body Weight Ratio**		**Epididymides to Brain Weight Ratio**	
Control	1.26	0.17	0.00211	0.00027	0.56	0.06
500 mg/kg	0.70	0.17	0.00121	0.00031	0.31	0.08
P-Value	p = .000		p = .000		p = .000	

Dose Group	Heart Unadjusted Weight		Heart to Body Weight Ratio		Heart to Brain Weight Ratio	
Control	2.00	0.17	0.00337	0.00031	0.89	0.06
500 mg/kg	1.95	0.13	0.00336	0.00022	0.87	0.05
P-Value	p = .444		p = .961		p = .382	
Dose Group	**Kidneys Unadjusted Weight**		**Kidneys to Body Weight Ratio**		**Kidneys to Brain Weight Ratio**	
Control	3.81	0.26	0.00641	0.00044	1.70	0.08
500 mg/kg	3.76	0.23	0.00649	0.00052	1.68	0.09
P-Value	p = .629		p = .709		p = .639	
Dose Group	**Liver Unadjusted Weight**		**Liver to Body Weight Ratio**		**Liver to Brain Weight Ratio**	
Control	21.14	2.70	0.03535	0.00247	9.41	1.03
500 mg/kg	19.76	2.50	0.03388	0.00201	8.80	0.80
P-Value	p = .251		p = .162		p = .157	
Dose Group	**Spleen Unadjusted Weight**		**Spleen to Body Weight Ratio**		**Spleen to Brain Weight Ratio**	
Control	1.08	0.21	0.00181	0.00026	0.48	0.08
500 mg/kg	1.14	0.21	0.00195	0.00029	0.51	0.08
P-Value	p = .568		p = .255		p = .516	
Dose Group	**Testes Unadjusted Weight**		**Testes to Body Weight Ratio**		**Testes to Brain Weight Ratio**	
Control	3.59	0.27	0.00605	0.00053	1.60	0.12
500 mg/kg	2.15	0.33	0.00372	0.00063	0.96	0.15
P-Value	p = .000		p = .000		p = .000	
Dose Group	**Thymus Unadjusted Weight**		**Thymus to Body Weight Ratio**		**Thymus to Brain Weight Ratio**	
Control	0.48	0.09	0.00080	0.00013	0.21	0.04
500 mg/kg	0.47	0.08	0.00081	0.00014	0.21	0.03
P-Value	p = .745		p = .928		p = .781	

N = 10 for the control and 500 mg/kg dose groups for all measures

F. Sperm Count

There were statistically significant sperm count differences between the control group and 500 mg/kg dose group (p-value = .000.) The 500 mg/kg dose group had a much lower sperm count per sample.

Table 17. Satellite/Recovery Male Sperm Counts		
Dose Group	**Sperm Count/ Sample (million/gram)**	
	Mean	**SD**
Control	108.78	52.62
500 mg/kg	31.13	16.34
P-value	p = .000	

N = 10 for the control and 500 mg/kg dose groups

G. Prothrombin Time

Prothrombin times were very similar between the two dose groups as evidenced by a t-test p-value well above the significance level of .05.

Table 18. Satellite/Recovery Male Prothrombin Time (seconds)		
Dose Group	**Mean**	**SD**
Control	9.58	0.41
500 mg/kg	9.55	0.57
P-value	p = 0.876	

N = 10 for the control and 500 mg/kg dose groups

IV. Results – Females

A. Clinical Chemistry

The only significant difference in female clinical chemistry results was in inorganic phosphorus (PHOS) where the 500 mg/kg dose had a higher mean PHOS level than the 125 mg/kg dose.

Table 19. Female Clinical Chemistry Results

Dose Group	N	ALB		ALKP		ALT		AST	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	8	3.18	0.25	155.50	79.93	88.75	15.51	105.00	23.46
31.25 mg/kg	9	3.17	0.37	152.22	62.20	106.00	49.40	99.78	27.37
125 mg/kg	10	3.23	0.43	135.00	32.62	88.00	24.29	106.90	36.29
500 mg/kg	8	3.39	0.14	128.38	64.17	89.25	23.15	91.88	20.21
P-Value		K-W p = .441		Lognormal p = .834		Lognormal p = .718		Lognormal p = .687	

Dose Group	N	BUN		CA		CHOL		CREA	
Control	8	13.50	6.23	11.88	0.65	74.13	15.79	0.50	0.17
31.25 mg/kg	9	16.78	4.58	11.98	0.58	78.44	21.61	0.50	0.11
125 mg/kg	10	14.30	5.48	11.77	0.51	63.80	13.81	0.56	0.12
500 mg/kg	8	14.5	4.41	12.18	0.25	70.00	9.47	0.54	0.11
P-Value		p = .598		p = .430		p = .247		p = .688	

Dose Group	N	GLOB		GLU		LDH		PHOS	
Control	8	3.04	0.20	153.13	61.18	717.25	818.23	11.65	1.83
31.25 mg/kg	9	2.79	0.29	162.00	54.34	431.78	188.40	12.00	1.83
125 mg/kg	10	2.84	0.16	137.50	37.03	693.50	819.43	11.25	0.92
500 mg/kg	8	2.89	0.19	144.00	24.27	418.75	121.92	13.38	1.10
P-Value		p = .124		p = .688		K-W p = .898		p = .031 500 mg/kg > 125 mg/kg (p=.022)	

Dose Group	N	TBIL		TP	
Control	8	0.46	0.52	6.21	0.45
31.25 mg/kg	9	0.40	0.28	5.94	0.55
125 mg/kg	10	0.26	0.21	6.08	0.49
500 mg/kg	8	0.26	0.15	6.26	0.27
P-Value		Lognormal p = .486		p = .485	

Dose Group	N	Cl		Na		K	
Control	8	105.38	1.85	149.75	1.83	8.99	1.46
31.25 mg/kg	9	104.56	1.81	148.56	0.88	9.59	1.15
125 mg/kg	10	104.6	2.88	150.7	2.31	9.44	0.99
500 mg/kg	7*	106.29	2.06	149.29	2.69	10.40	2.24
P-Value		p = .389		p = .154		p = .329	

* One rat did not have Cl, Na, or K measures.

B. Hematology

The only significant difference in female hematology was in the percent basophils (BASO %) where the mean percent at the 500 mg/kg dose was significantly greater than the mean percent at the 125 mg/kg dose.

Table 20. Female Hematology Results

Dose Group	N*	NEU (K/uL)		NEU (%N)		LYM (K/uL)		LYM (%L)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	8	3.88	1.99	29.50	9.72	7.42	3.57	57.80	12.65
31.25 mg/kg	8	2.82	0.98	27.70	10.27	6.78	3.48	60.74	14.84
125 mg/kg	10	2.99	2.79	26.91	11.26	5.78	1.96	62.59	12.81
500 mg/kg	7	3.67	1.66	35.54	12.59	4.77	1.64	46.66	9.49
P-Value		Lognormal p = .466		p = .423		p = .287		p = .087	

Dose Group	N*	MONO (K/uL)		MONO (%M)		EOS (K/uL)		EOS (%E)	
Control	8	0.91	0.43	8.21	6.97	0.06	0.04	0.42	0.20
31.25 mg/kg	8	0.70	0.30	7.22	3.53	0.04	0.01	0.84	1.17
125 mg/kg	10	0.74	0.40	7.47	2.62	0.04	0.02	0.41	0.23
500 mg/kg	7	1.21	0.82	11.29	4.77	0.11	0.14	0.56	0.29
P-Value		Lognormal p = .351		Lognormal p = .246		Lognormal p = .195		Lognormal p = .454	

Dose Group	N*	BASO (K/uL)		BASO (%B)		RBC (M/uL)		HGB (g/dL)	
Control	8	0.52	0.35	4.08	2.35	6.82	0.42	13.99	0.85
31.25 mg/kg	8	0.38	0.24	3.92	2.51	7.07	0.65	14.15	1.42
125 mg/kg	10	0.29	0.26	2.62	1.31	7.00	0.56	14.29	1.19
500 mg/kg	7	0.57	0.14	5.95	2.20	6.94	0.43	14.47	0.71
P-Value		p = .124		p = .028 500 mg/kg > 125 mg/kg (p=.015)		p = .813		p = .849	

Dose Group	N*	HCT (%)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
Control	8	37.98	3.16	55.61	2.40	20.50	0.74	36.88	1.13
31.25 mg/kg	8	38.64	3.40	54.65	1.17	20.00	0.35	36.56	0.99
125 mg/kg	10	39.66	4.05	56.59	2.46	20.42	0.58	36.10	1.30
500 mg/kg	7	38.83	2.41	55.97	1.30	20.90	1.58	37.33	2.58
P-Value		p = .770		p = .247		p = .293		p = .446	

Dose Group	N*	RDW (%)		PLT (K/uL)		MPV (fL)		WBC (K/uL)	
Control	8	15.98	0.90	983.63	276.46	5.24	0.23	12.80	4.97
31.25 mg/kg	8	15.90	1.04	999.00	242.48	5.24	0.44	10.70	3.52
125 mg/kg	10	17.07	1.57	1155.40	234.58	5.08	0.37	9.84	4.88
500 mg/kg	7	16.66	0.90	973.00	309.18	5.31	0.57	10.27	2.78
P-Value		p = .135		p = .418		p = .667		p = .502	

* There was no hematology data for one animal in the 31.25 mg/kg dose group and for one animal in the 500 mg/kg dose group.

C. Body Weight

Female body weights were recorded several times during the study. There were no significant differences in the body weights for any dose group during the pre-pregnancy period, the gestational period, or post-partum.

Table 21. Female Total Body Weight – Pre-Pregnancy

Dose Group	N	Pre-pregnancy Day 0		Pre-pregnancy Day 7		Pre-pregnancy Day 14	
		Mean	SD	Mean	SD	Mean	SD
Control	10	214.10	15.34	227.20	15.59	244.70	15.30
31.25 mg/kg	10	216.70	12.03	235.70	13.00	254.90	14.33
125 mg/kg	10	216.80	10.01	230.80	13.94	243.90	15.36
500 mg/kg	10	214.40	14.98	224.60	16.68	237.50	19.91
P-value	Repeated measures p = .437						

Table 22. Female Total Body Weight - Gestation

Dose Group	N	Gestational Day 0		Gestational Day 7		Gestational Day 14		Gestational Day 20	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	8	248.75	15.04	293.50	14.73	337.88	17.10	412.00	17.20
31.25 mg/kg	8*	258.25	20.02	300.88	27.50	342.38	31.89	415.75	39.71
125 mg/kg	10	255.30	12.37	303.90	16.13	348.20	19.06	420.10	28.55
500 mg/kg	7*	243.29	27.12	288.00	26.76	326.00	25.85	397.00	30.77
P-value	Repeated measures p = .376								

* Two animals had no sperm plug but were pregnant; no gestational weight was collected.

Table 23. Female Total Body Weight – Post-Partum

Dose Group	N	Post-partum Day 0		N	Post-partum Day 4	
		Mean	SD		Mean	SD
Control	8	300.25	36.98	7*	312.00	34.33
31.25 mg/kg	9	306.22	22.40	7*	318.43	23.27
125 mg/kg	10	311.80	39.17	10	320.60	36.05
500 mg/kg	8	302.00	31.46	7*	324.71	27.51
P-value	p = .884			p = .893		

* No post-partum weight data was collected on 4 animals.

During the 14 day pre-pregnancy period, the 500 mg/kg dose group gained less weight per week than the 31.25mg/kg dose group.

Table 24. Female Body Weight Gain – Pre-Pregnancy					
Dose Group	**N**	**Pre-pregnancy Day 7 - Day 0**		**Pre-pregnancy Day 14 - Day 7**	
		Mean	**SD**	**Mean**	**SD**
Control	10	13.10	8.02	17.50	4.88
31.25 mg/kg	10	19.00	7.35	19.20	6.27
125 mg/kg	10	14.00	8.34	13.10	6.42
500 mg/kg	10	10.20	9.99	12.90	7.81
P-value	Repeated measures p = .008 500 mg/kg < 31.25 mg/kg (p=.006)				

Table 25. Female Body Weight Gain - Gestation									
Dose Group	**N**	**Gestational Day 0 to Day 7**		**Gestational Day 7 to Day 14**		**Gestational Day 14 to Day 20**		**Gestational Day 20 to Delivery (Post-Partum Day 0)**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	8	44.75	6.45	44.38	5.13	74.13	5.67	-111.75	31.24
31.25 mg/kg	8*	42.63	9.23	41.50	7.19	73.38	13.95	-112.25	26.22
125 mg/kg	10	48.60	7.63	44.30	6.09	71.90	12.99	-108.30	31.21
500 mg/kg	7*	44.71	6.75	38.00	20.03	71.00	19.14	-99.86	17.55
P-value	Repeated measures p = .834								

* Two animals had no sperm plug but were pregnant; no gestational weight was collected.

Table 26. Female Body Weight Gain – Post-Partum			
Dose Group	**N**	**Post-partum Day 0 to Day4**	
		Mean	**SD**
Control	7*	8.14	14.04
31.25 mg/kg	7*	16.57	16.34
125 mg/kg	10	8.80	23.35
500 mg/kg	7*	16.00	12.73
P-value	p = .697		

* No post-partum weight gain data was collected on 4 animals.

D. Food Consumption

During the pre-pregnancy period, the total food consumption of the 500 mg/kg dose group was lower than the 31.25 mg/kg dose group, but otherwise, there were no differences among the other pre-pregnancy dose groups, during gestation, or during the first four post-partum days.

Table 27. Female Food Consumption – Pre-Pregnancy			
Dose Group	**N**	**Mean**	**SD**
Control	10	254.94	17.78
31.25 mg/kg	10	263.50	17.64
125 mg/kg	10	248.24	19.43
500 mg/kg	10	234.37	18.17
P-value		p = .009 500 mg/kg < 31.25 mg/kg (p=006)	

Table 28. Female Food Consumption - Gestation									
Dose Group	**N**	**Gestational Day 0 to Day 7**		**Gestational Day 7 to Day 14**		**Gestational Day 14 to Day 20**		**Gestational Day 20 to Delivery (Post-Partum Day 0)**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control	8	155.24	12.95	178.04	14.68	156.01	11.42	25.44	22.02
31.25 mg/kg	8*	158.04	16.43	173.40	19.68	153.25	16.51	21.55	11.11
125 mg/kg	9**	165.17	18.23	183.53	19.36	164.91	16.59	32.37	25.03
500 mg/kg	7*	153.57	15.96	168.70	27.30	142.96	23.32	31.34	15.46
P-value	Repeated measures p = .207								

* Two animals had no sperm plug but were pregnant; no gestational weight was collected.

** Animal was missing one data point and was not included in the repeated measures analysis.

Table 29. Female Food Consumption – Post-Partum			
Dose Group	**N**	**Mean**	**SD**
Control	7*	94.60	35.95
31.25 mg/kg	7*	107.09	18.83
125 mg/kg	10	96.91	44.87
500 mg/kg	7*	102.94	28.57
P-value		p = .899	

* No post-partum food consumption data was collected on 4 animals.

E Organ Weight

1. Non-Uterus

There were significant differences in female organ weight metrics for brain and spleen. Specifically, the mean brain weight, accounting for terminal body weight, of the 31.25 mg/kg dose group was significantly less than the control group. For spleen weight, accounting for terminal body weight and for spleen to body-weight ratio, both the 31.25 mg/kg dose group and the 500 mg/kg dose group had significantly lower means than did the control group.

Table 30. Female Organ Weight Results

Dose Group	N	Adrenals Unadjusted Organ Weight		Adrenals to Body Weight Ratio		Adrenals to Brain Weight Ratio	
		Mean	SD	Mean	SD	Mean	SD
Control	8	0.087	0.015	0.00030	0.00007	0.0437	0.0077
31.25 mg/kg	9	0.081	0.019	0.00027	0.00006	0.0422	0.0096
125 mg/kg	10	0.098	0.026	0.00033	0.00010	0.0493	0.0136
500 mg/kg	7*	0.080	0.014	0.00026	0.00005	0.0398	0.0072
P-Value		Lognormal p = .216		p = .225		Lognormal p = .258	

Dose Group	N	Brain Unadjusted Organ Weight		Brain to Body Weight Ratio		Brain to Brain Weight Ratio
Control	8	2.01	0.072	0.00684	0.00065	Not applicable
31.25 mg/kg	9	1.92	0.055	0.00636	0.00042	
125 mg/kg	10	1.99	0.084	0.00666	0.00069	
500 mg/kg	8	1.99	0.088	0.00663	0.00042	
P-Value		p = .020 31.25 mg/kg < Control (p=.024)		Lognormal p = .371		

Dose Group	N	Heart Unadjusted Organ Weight		Heart to Body Weight Ratio		Heart to Brain Weight Ratio	
Control	8	1.18	0.162	0.00397	0.00021	0.5865	0.0724
31.25 mg/kg	9	1.16	0.137	0.00381	0.00028	0.6020	0.0644
125 mg/kg	10	1.21	0.190	0.00399	0.00033	0.6047	0.0825
500 mg/kg	8	1.16	0.161	0.00386	0.00041	0.5838	0.0670
P-Value		p = .514		p = .563		p = .903	

Dose Group	N	Kidneys Unadjusted Organ Weight		Kidneys to Body Weight Ratio		Kidneys to Brain Weight Ratio	
Control	8	2.06	0.134	0.00704	0.00089	1.0274	0.0566
31.25 mg/kg	9	2.03	0.254	0.00667	0.00056	1.0538	0.1159
125 mg/kg	10	2.14	0.234	0.00715	0.00100	1.0761	0.1320
500 mg/kg	8	2.04	0.216	0.00680	0.00065	1.0259	0.0692
P-Value		p = .617		p = .576		p = .685	

Dose Group	N	Liver Unadjusted Organ Weight		Liver to Body Weight Ratio		Liver to Brain Weight Ratio	
Control	7*	12.32	1.818	0.0406	0.00372	6.085	0.7944
31.25 mg/kg	9	12.10	1.905	0.0398	0.00413	6.289	0.8773
125 mg/kg	10	11.71	1.356	0.0388	0.00250	5.879	0.6732
500 mg/kg	8	11.19	1.082	0.0372	0.00153	5.637	0.4927
P-Value		p = .217		p = .181		p = .307	

Dose Group	N	Ovaries Unadjusted Organ Weight		Ovaries to Body Weight Ratio		Ovaries to Brain Weight Ratio	
Control	8	0.154	0.033	0.00054	0.00017	0.0774	0.0184
31.25 mg/kg	9	0.144	0.024	0.00048	0.00008	0.0752	0.0127
125 mg/kg	10	0.153	0.021	0.00052	0.00011	0.0768	0.0108
500 mg/kg	8	0.142	0.035	0.00047	0.00011	0.0716	0.0170
P-Value		p = .788		p = .676		p = .856	

Dose Group	N	Spleen Unadjusted Organ Weight		Spleen to Body Weight Ratio		Spleen to Brain Weight Ratio	
Control	7*	0.739	0.150	0.00243	0.00036	0.3653	0.0712
31.25 mg/kg	9	0.618	0.082	0.00204	0.00022	0.3215	0.0402
125 mg/kg	10	0.629	0.088	0.00209	0.00025	0.3166	0.0476
500 mg/kg	8	0.582	0.103	0.00193	0.00024	0.2932	0.0484
P-Value		p = .008 31.25 mg/kg < Control (p=.035) 500 mg/kg < Control (p=.008)		p = .007 31.25 mg/kg < Control (p=.031) 500 mg/kg < Control (p=.005)		p = .079	

Dose Group	N	Thymus Unadjusted Organ Weight		Thymus to Body Weight Ratio		Thymus to Brain Weight Ratio	
Control	8	0.318	0.112	0.00106	0.00030	0.1579	0.0530
31.25 mg/kg	9	0.259	0.095	0.00087	0.00036	0.1354	0.0514
125 mg/kg	10	0.255	0.100	0.00083	0.00028	0.1271	0.0483
500 mg/kg	8	0.261	0.068	0.00087	0.00024	0.1315	0.0344
P-Value		p = .370		p = .408		p = .560	

* One animal in the 500 mg/kg dose group was missing adrenal information and one animal in the control group was missing liver and spleen information.

2. Uterus

There was an outlier in the uterus data that affected the ability to analyze the data. One uterus weight in the 500 mg/kg dose group was nearly two times that of the others in that dose group.

This affected normality such that ANOVA could not be performed for the uterus to body weight and uterus to brain weight analyses and a Kruskal-Wallis non-parametric test was used. For the raw data, a natural log transformation brought the uterus weights nearer to normality; however when the body

weight was accounted for, the residuals were not normal (p = .001). Results are shown using the transformed data, keeping the outlier in the data set, but *should be interpreted with extreme caution*.

Results are also shown excluding the outlier. With the removal of the outlier, all the uterus data passed normality assumptions and ANOVAs/ANCOVA were performed. Results showed that the for both the organ weight, accounting for terminal body weight, and for the uterus to brain weight ratio, the 500mg/kg means were significantly less than the 125 mg/kg means. Including the outlier masked these differences.

Table 31. Female Uterus Weight Results

Dose Group	N	Uterus Unadjusted Organ Weight		Uterus to Body Weight Ratio		Uterus to Brain Weight Ratio	
Control	8	0.669	0.100	0.00230	0.00051	0.3337	0.0468
31.25 mg/kg	9	0.659	0.124	0.00217	0.00037	0.3425	0.0599
125 mg/kg	10	0.752	0.106	0.00253	0.00052	0.3783	0.0577
500 mg/kg	8	0.686	0.296	0.00229	0.00104	0.3467	0.1544
P-Value		Lognormal p = .436		K-W p = .196		K-W p = .096	

Dose Group	N	Uterus Unadjusted Organ Weight without Outlier		Uterus to Body Weight Ratio without Outlier		Uterus to Brain Weight Ratio without Outlier	
Control	8	0.669	0.100	0.00230	0.00051	0.3337	0.0468
31.25 mg/kg	9	0.659	0.124	0.00217	0.00037	0.3425	0.0599
125 mg/kg	10	0.752	0.106	0.00253	0.00052	0.3783	0.0577
500 mg/kg	7	0.586	0.095	0.00190	0.00023	0.2941	0.0440
P-Value		p = .035 500 mg/kg < 125 mg/kg (p=.027)		p = .060		p = .028 500 mg/kg < 125 mg/kg (p.=016)	

F Prothrombin Time

An outlier in the control group prevented the data from meeting the assumptions of normality. One Prothrombin time was approximately 65% greater than the other data points in the group. Transformation did not bring the data into normality, therefore a Kruskal-Wallis test was used for analysis. There was no significant difference among the dose groups. If the outlier was removed, data met assumptions for ANOVA and results also showed no significant differences.

Table 32. Female Prothrombin Time Results

Dose Group	N	With Outlier		N	Outlier Removed	
		Mean	SD		Mean	SD
Control	8	9.56	2.07	7	8.84	.41
31.25 mg/kg	9	9.18	.38	9	9.18	.38
125 mg/kg	10	9.08	.37	10	9.08	.37
500 mg/kg	8	9.43	.59	8	9.43	.59
P-Value		K-W p = .399			p = .103	

VI. Reproductive Data

There were no discernible differences among the dose group on any of the reproductive endpoints. Sample sizes (n=10) were too small and proportions (often 8 to 10 out of 10) were too similar to allow for statistical comparison of the count distributions.

Table 33. Reproductive Summary Measures

Reproductive Measures	Dose Group			
	Control	31.25 mg/kg	125 mg/kg	500 mg/kg
Females showing evidence of copulation (N)	9	9	10	9
Females achieving pregnancy (N)	8	9	10	8
Dams with live young born (N)	8	9	10	8
Dams with live young at day 4 pp (N)	6	9	9	7

N-total = 10 for each dose group within each reproductive measure

VII. Litter Measures

Minimal and inconsistent differences were found among the four dose groups for the twelve litter measures. These minimal differences within all litter information measures were illustrated by p-values well above a statistically significant level.

Table 34. Litter Information Measures

Litter Information Measure	P-value
Pregnancy Length	0.796*
Days for Conception	0.52*
Live Pups at Birth	0.974*
Live Pups at Day 4	0.724*
Sex Ratio at Birth	0.458+
Sex Ratio at Day 4	0.381+
Avg. Pup Weight per Litter at Birth	0.525
Avg. Pup Weight per Litter at Day 4	0.898
Pup Abnormalities at Birth	0.573*
Pre-Implant Loss	0.575*
Pre-Natal Loss	0.744*
Post-Natal Loss	0.431*

* Analyzed using Non-parametric K-W test

+ Data was natural Log transformed to fit ANOVA assumptions.

Appendix Q

Study Protocol with Modifications

ANIMAL USE PROTOCOL
TOXICOLOGY PORTFOLIO
ARMY INSTITUTE OF PUBLIC HEALTH
U.S. ARMY PUBLIC HEALTH COMMAND
ABERDEEN PROVING GROUND, MD 21010-5403

PROTOCOL TITLE: Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat.

PROTOCOL NUMBER: ØFP4-93-12-Ø3-Ø3

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I. NON-TECHNICAL SYNOPSIS

The repeated-dose toxicity as well as the reproductive/developmental toxicity of 3-nitro-1,2,4-triazol-5-one (NTO) will be evaluated in the rat according to guidelines set forth by the Organisation for Economic Co-operation and Development (OECD). NTO is an insensitive, energetic material used in a variety of explosive formulations currently in

various stages of development and production. Recent toxicity testing has revealed that repeated exposures to higher concentrations of NTO may induce testicular toxicity and affect sperm production in male rats. Due to these recent findings and the increasing popularity of NTO as an effective insensitive munition, the US Environmental Protection Agency (EPA) has requested further studies to address these concerns. Briefly, groups of 20 adult rats will be exposed to NTO orally every day for a minimum of 28 days both prior to and during a period of time where males and females will be co-housed with the intent to reproduce. Male adult rats will be euthanized and necropsied at the end of the co-housing period while female adult rats will continue to be dosed through post-partum day 3 or 4. Reproduction will be assessed by the number of pregnant females at the end of the co-housing period while development will be assessed by a careful examination of the litters on post-partum day 4. The repeated-dose toxicity of NTO on adult rats will be determined through changes in body weights, food consumption patterns (when applicable), and daily observations during dosing as well as a review of organ weights and histopathological evaluations of harvested tissues. In addition, a separate group of 20 adult male rats will be added as a satellite recovery group to determine the possible reversibility of NTO's effects on the reproductive system in male rats. These animals will be dosed concurrently with the main study animals for a period of 4 weeks. These recovery animals will then be held for a period not to exceed 4 weeks, euthanized, and necropsied .

II. BACKGROUND

II.1. Background: 3-Nitro-1,2,4-triazol-5-one (NTO) is being investigated as a less sensitive direct replacement for traditional explosives such as TNT and RDX. NTO is a crystalline powder that is one of the components used in the formulation of an insensitive explosive referred to as IMX101. NTO was first reported in 1905, but was not used as an explosive until the early 1980's when it was discovered that the French were developing a "new insensitive explosive", which was later reported to be NTO. Renewed interest in the energetic properties of NTO has been fueled by the need to develop munitions that are less prone to inadvertent initiation during transport and routine handling. The reduced sensitivity to environmental stimuli and nearly equal performance during testing make NTO-based formulations desirable replacements for currently fielded munitions (references 1 and 2). As a potential new component of munitions formulations, NTO must not only meet certain performance criteria, but must also be acceptable from the perspective of human health and the environment. To ensure its safe use by military personnel and production employees handling the material on a daily basis, the toxicity of NTO must be investigated. Toxicological testing will be conducted by the U.S. Army Institute of Public Health (USAIPH), Portfolio of Toxicology (TOX).

Both 14- and 90-day repeated dose oral toxicity studies were previously performed by this Institute. In the 14-day repeated-dose study, testes weights and weight ratios were significantly reduced compared to controls in male rats administered 500 mg/kg-day NTO and above. No significance was observed for epididymis weights or weight ratios compared to controls and histopathology was not performed on any tissues from the 14-day study. The subchronic study on NTO revealed significant changes in both testes

and epididymide weights and weight ratios at dosages of 315 mg/kg-day and above. Significant reductions in sperm counts were also noted at dosages of 315 mg/kg-day and above. Histopathology performed on the 90-day tissues revealed significant incidences of testicular hypoplasia at dosages of 315 mg/kg-day and above as well as insignificant, less severe, testicular hypoplasia at dosages of 100 mg/kg-day and below (reference 3). The proposed study will not only allow investigators to directly evaluate NTO's possible effects on reproduction, but will also allow for a more complete histopathological evaluation of the male reproductive organs in an attempt to determine a cause for the toxicity observed in the previous studies.

II.2. Literature Search for Duplication

II.2.1. Literature Source(s) Searched: BRD (Biomedical Research Database), DOAC (DTIC Online Access Controlled)* Technical Reports, DOAC Research in Progress, FEDRIP, PubMed, Web of Science

II.2.2. Date of Search: 12 August 2011(updated 10 January 2012)

II.2.3. Period of Search: 1898-2012

II.2.4. Key Words of Search: (3-nitro-1,2,4-triazol-5-one or 3 nitro 1,2,4 triazol 5 one or triazole* or nitro compound*) and toxic* and (reproduc* or develop* or growth* or embryo* or fetus or fetal) and (repeat* dos* or time factor*) and (((cardiac near blood near collect*) or (heart near blood near collection*)) and (method* or technic* or techniq*)) and (rat or rats)

II.2.5. Results of Search: A total of 127 references resulted from the literature search that was performed for NTO. However, no reproductive/developmental toxicity studies for NTO were found that would suggest that this study would be a duplicate effort. As such, the present study is not a duplication of the information available in the literature.

III. OBJECTIVE/HYPOTHESIS

The primary objective of this study is to determine the initial reproductive and developmental toxicity of NTO through the use of a screening test. This test does not provide complete information on all aspects of reproduction and development and only offers limited means of detecting postnatal manifestations of prenatal exposure or effects induced during postnatal exposure. The secondary objective of this study will be to confirm the effects of repeated-dose exposure to NTO using different exposure durations and dose levels than previously evaluated.

IV. MILITARY RELEVANCE

As a result of an initiative by the Department of Defense (DOD) to improve munitions safety, the US Army is developing insensitive munitions (IM) for incorporation into its inventory of conventional military munitions systems. The Army's IM Program is dedicated to developing munitions that reliably perform as they are intended but are

less prone to inadvertent initiation from external stimuli such as bullet/fragment impact, heat from fire, and shock from neighboring explosions (reference 4). The production of insensitive munitions requires the use of intrinsically less sensitive explosives that contribute to lower order responses to inadvertent external stimuli. Despite the slightly lower performance of NTO compared to TNT, there has been a renewed interest in NTO use in explosive formulations based on its lower sensitivity as a melt-cast medium observed during testing and the less stringent shipping requirements. This has led to the development of a range of melt-castable explosives at Picatinny Arsenal, collectively known as "PAX" explosives (reference 5). To support possible fielding of these PAX explosives, additional reproductive/developmental toxicity data in a mammalian system needs to be generated to assess the occupational health hazards associated with the use and production of this material.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This study consists of a repeated-dose oral toxicity study as well as a reproductive/developmental screening test. Groups of 10 male and 10 female rats will be dosed daily with NTO via oral gavage for 2 weeks prior to co-housing and for an additional 2 weeks during co-housing. Rats will be monitored throughout these 4 weeks for body weight, food consumption (except during co-housing), and clinical signs. At the conclusion of the 4 weeks, adult male rats will be anesthetized for blood collection, euthanized, and necropsied. Selected tissues from the male rats will be submitted for histopathological evaluation. Adult female rats will continue to be dosed with NTO through day 3 or 4 post-partum and monitored for body weight, food consumption, and clinical signs. They will be anesthetized for blood collection, euthanized, and necropsied with selected tissues submitted for histopathological evaluation. Pups from each litter will be counted, sex determined, and weighed within 24 hours of parturition and will be observed for any abnormal behavior. Dead pups and pups euthanized on day 4 post-partum will be carefully examined externally for gross abnormalities.

V.1.1. Repeated-Dose Toxicity and Reproductive/Developmental Screening Test

This study will be conducted in a manner consistent with the methods outlined in OECD Test Guideline 422 (reference 6). The route of administration will be by oral gavage with pure NTO suspended/dissolved in an appropriate vehicle (e.g. corn oil). Forty adult Sprague Dawley rats of each sex (N=80) will be randomly distributed into 3 dose groups and a vehicle control group (10 rats of each sex per dose group). It is expected that this number of animals will produce enough pregnancies and offspring to ensure a meaningful evaluation of the potential of NTO to affect fertility, pregnancy, maternal and suckling behavior, and initial growth and development of the F_1 offspring from conception to day 4 post-partum. Rats will be exposed daily to control, 31.25, 125, or 500 mg/kg-day NTO 7 days/week for a maximum of 56 days. The highest treatment level is based primarily on the reproductive effects observed in male rats following a subchronic oral study performed by this Institute (reference 3). Based on this Institute's

experience with previous animal shipments involving underweight/young rats, 2 additional rats of each sex will be ordered to ensure the study is initiated using the required number of rats in each dose group. Rats used for weight matching purposes but not placed on study will either be transferred to another protocol or humanely euthanized.

In addition to the main study, 20 male rats will be added to serve as satellite recovery groups for the highest dose group and the control group. From this group of 20 rats, 10 will serve as diluent controls and 10 will be dosed with the highest concentration of NTO given to rats in the main study. These animals will be dosed concurrently with the main study animals for the appropriate time period and held for a period of at least 14 days (not to exceed one month) following cessation of dosing. Animals used as satellite/recovery animals will not be co-housed but will be dosed with NTO for the same time period as the main study animals, held for a 14-28-day recovery period, and then anesthetized, bled, and necropsied with selected tissues sent for histopathological evaluation. The purpose of the satellite group is to evaluate the reversibility, persistence, or delayed occurrence of the toxic effects associated with repeated exposure to NTO observed during the previous studies performed by this Institute.

Dosing of both sexes will begin 2 weeks prior to co-housing following an acclimatization period of no less than 5 days. Dosing is continued in both sexes during the 2 week co-housing period. Male rats will continue to be dosed with NTO until the minimum dosing period of 28 days has been completed regardless of how quickly a successful mating is verified. Following the 28-day dosing period, male rats will be anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation. Anesthesia, cardiac blood sampling, and necropsy procedures are described in Sections V.4.1.2.1, V.1.8, and V.4.6, respectively. Female rats showing no evidence of copulation (presence of sperm plug) following the 2-week co-housing period will continue to be dosed and euthanized 24-26 days after the last day of the co-housing period. These animals will also be anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation. Daily dosing of the successfully-bred females will continue throughout pregnancy and at least up to, and including, day 3 post-partum or the day before euthanasia. In order to allow for overnight fasting of dams prior to blood collection, the pups may be euthanized on post-partum day 4 with euthanasia of the dams occurring the next day. Gross external examinations of all pups will occur following euthanasia. Parental females will also be anesthetized, bled, and necropsied with selected tissues collected, weighed, and submitted for histopathological evaluation.

Repeated-Dose Toxicity and Reproductive/Developmental Screening Test

Group	No. of Male Rats	No. of Female Rats
Control	10	10
31.25 mg/kg NTO	10	10
125 mg/kg NTO	10	10
500 mg/kg NTO	10	10
Weight Matching	2	2
Satellite/Recovery	20	0
Pups*	200	200
	TOTAL = 262	**TOTAL = 242**

(*) The estimation of numbers of pups was made with the assumptions that each pair would produce an average of 10 pups and the sex ratio of the offspring would be 1:1.

V.1.2. Test Substance: This study will be conducted with 3-nitro-1,2,4-triazol-5-one (NTO). A sample of this test substance was supplied by BAE SYSTEMS, Ordnance Systems, Kingsport, TN for use in a previously conducted subchronic oral toxicity study in rats. The test sample was received at the test facility in November 2008 and is identified as Batch# 10NTO7-3 and Lot# BAE07B305-001. If it is determined that enough of this batch of test material is adequate to complete this study, then it will be used. If additional sample(s) of the test substance may be required from BAE SYSTEMS, appropriate identification documentation will be made in the study records. To facilitate the oral gavage procedure, NTO will be mixed with an appropriate diluent. Once an appropriate diluent has been determined, the pH of each NTO suspension/solution will be verified to ensure that the test material is not corrosive. Samples of each batch of the resulting solutions/suspensions will be submitted to the USAIPH Laboratory Sciences(LS) portfolio for concentration verification. In addition, appropriate samples will be submitted to verify the stability and homogeneity of NTO in the diluent IAW LS SOP DLS 801 (reference 7).

Test Substance Chemical/Physical Properties

Name	3-nitro-1,2,4-triazol-5-one
Synonym	NTO
CAS#	932-64-9
Physical State	White to pale yellow crystalline powder
Molecular Formula	$C_2H_2N_4O_3$
Molecular Weight	130
Density	1.93 g/cm^3
Solubility	Soluble in water (16 g/L)
Purity (by HPLC)	99.6%

V.1.3. Administration of Test Substance by Oral Gavage Dose: Oral dosing/gavage will be performed using a stainless steel 16 ga x 2 inch gavage needle. As per OECD Test Guidelines, the volume given will not exceed 10 ml/kilogram of body weight (reference 6). Animals in the control group will receive the vehicle in the highest volume used for the test groups.

V.1.4. Dose Selection for Oral Gavage: Dose selection for the rats is based on the findings from 14- and 90-day oral studies that were performed previously by this Institute (reference 3). The doses for this study were selected based primarily on the results of the 14-day study with the expectation of producing reproductive effects at the highest dose of 500 mg/kg-day. The lower doses were then calculated using the OECD guideline-recommended four-fold interval.

V.1.5. Co-Housing Procedure: A 1:1 (one male to one female) co-housing procedure will be used in this study with the exception of the unlikely incidence of pre-term deaths of male rats. Female rats will be housed with the same male rat continuously until the presence of a vaginal plug is noted or until 2 weeks have elapsed—whichever comes first. Female rats will be examined for the presence of a vaginal plug prior to dosing each morning during the co-housing period. Day 0 of pregnancy is defined as the day the vaginal plug is observed.

V.1.6. Observations: A thorough physical examination of each rat will be performed by study personnel at a similar time at least once per day. The examination process will consist of each rat being removed from its home cage, individually handled, and carefully observed. Observations will include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or abnormal behavior (e.g., self mutilation, walking backwards). All data related to the observation of rats will be detailed and thoroughly documented in the study records by study personnel.

The duration of gestation will be recorded and is calculated from day 0 of pregnancy as indicated by the presence of a vaginal plug. Each litter will be examined as soon as possible after delivery to establish the number and sex of pups, stillbirths, live births, runts, and the presence of gross abnormalities. Live pups will be counted and their sex and weight determined within 24 hours of parturition (day 0 or 1 post-partum) and on day 4 post-partum. In addition to the observation of parental animals, any abnormal behavior of the offspring will also be recorded.

V.1.7. Body Weight and Food Consumption: Male and female rats will be weighed on the first day of dosing, weekly thereafter, and at termination (pre-fasted and fasted). During pregnancy, female rats will be weighed on days 0, 7, 14, 20, within 24 hours of parturition, and day 4 post-partum. Food consumption will be monitored weekly during pre-mating, pregnancy, and lactation. Food consumption will not be monitored during the 2-week co-housing period. Food consumption will be monitored weekly for all recovery animals.

V.1.8. Hematology and Clinical Chemistry: Blood samples will be taken from all parental male and female rats just prior to necropsy. Anesthesia procedures are described in V.4.1.2.1. Once the anesthetic has taken effect (ensured by toe pinch), the

rat will be placed in dorsal recumbency. The rat can then be immobilized by either holding the base of the tail or by holding the forelimbs apart and upward with the thumb and index finger. There should be no response by the rat to entry of the needle into its skin. If there is any response, the rat is not at a deep enough level of anesthesia for this method of blood collection and the procedure will stop until the rat is anesthetized to a deeper plane of anesthesia. Following collection of the blood sample, the needle should be slowly withdrawn from the rat. To minimize blood hemolysis, the needle should be removed from the syringe, the microtube top removed, and the blood sample discharged directly into the microtube tube directly from the syringe. The microtube will then be recapped. EDTA tubes need to be inverted gently several times immediately after introducing blood into the tube. Male rats will be bled at the conclusion of the 2-week co-housing period and female rats will be bled on day 4 or 5 post-partum. Blood will be collected from the satellite/recovery animals at the conclusion of the recovery period (14 days – 1 month). For hematology samples, approximately 1.2 ml of blood will be transferred to an EDTA microtube and evaluated for total red blood cell and white blood cell counts, packed cell volume, hemoglobin, and five-part differential. For clinical chemistry samples, approximately 1.2 ml of blood will be transferred to a serum-gel microtube and evaluated for the following chemistries: BUN, CREA, GLU, TP, ALB, ALT, ALK P, AST, GLOB, CHOL, LDH, TBIL, CA, PHOS, and electrolytes. A portion of the blood from each animal (approx. 1.2 ml) will also be transferred to a sodium citrate microtube for analysis of prothrombin time. Details concerning clinical chemistry and hematology parameters are outlined in TOX SOP 034 and TOX SOP 001, respectively (references 8 and 9). At the discretion of management and/or the study director, the remainder of each blood sample may be transferred to an appropriate microtube for met-hemoglobin and sex hormone analysis. All animals will be fasted overnight prior to blood sampling.

V.1.9. Gross Necropsy: Euthanasia procedures are described in section V.4.6. All adult animals in the study will be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. Special attention will be paid to the organs of the reproductive system. All gross pathology changes will be recorded on CHPPM Form 333 or 23. If a necropsy cannot be performed immediately after a deceased animal is discovered, appropriate measures will be taken to ensure the animal is dead, and the animal will be refrigerated at temperatures low enough to minimize autolysis. The following organs and tissues, or representative samples, will be preserved in a suitable medium for possible future histopathological examination: all gross lesions; brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex); pituitary; thyroid parathyroid; thymus; lungs and trachea; pharynx; larynx; nose; heart; bone marrow (either femur, sternum or rib at the costochondral junction); salivary glands; liver; spleen; kidney; adrenals; pancreas; testes; uterus; aorta; esophagus; stomach; duodenum; jejunum; ileum; caecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; trachea; sternum with bone marrow; mammary gland; thigh musculature; eyes; femur (including articular surface); spinal cord at three levels (cervical, midthoracic, and lumbar) and exorbital lachrymal glands. In addition, the following organs will be weighed: liver, kidneys, adrenals, gonads,

spleen, brain, epididymides, uterus, thymus and heart. This tissue list may be altered at the discretion of the study staff based on observed toxicity and gross pathology findings. Prior to being weighed, organs will be carefully dissected and trimmed to remove fat and other tissue in a uniform manner. Animals in the satellite groups, if used, will undergo a full necropsy using the procedures described above approximately 14 days to one month after the completion of dosing. Formalin fixation is not recommended for routine examination of testes and epididymides. An alternative acceptable method such as Bouin's or Modified Davidson's fixative will be used for these tissues. Dead pups and pups euthanized at day 4 post-partum, or shortly thereafter, will, at least, be carefully examined externally for gross abnormalities.

V.1.10. Histopathology: Full histopathology will be carried out on the testes, epididymides, ovaries, and any gross lesions recognized of the animals in all dose groups with special emphasis on the stages of spermatogenesis in the male gonads and interstitial testicular cell structure.

V.1.11. Study Conduct: This study will be conducted in a manner consistent with the principles of 40 CFR (Code of Federal Regulations) Part 792 "Toxic Substance Control Act" (TSCA) Good Laboratory Practice (GLP) Regulation (reference 10). All study records will be made available to oversight organizations such as the Environmental Protection Agency as needed. The investigators and technicians will adhere to The Guide for Care and Use of Laboratory Animals, 2011 (reference 11).

Records will be kept in standard USAPHC laboratory notebooks and/or three ring binders. Daily records will be kept on survival and clinical signs collected on the animals during the exposure and recovery periods. Procedures for preparation of any euthanasia solution, drug administration, animal blood collection, observation logs, morbidity/mortality logs, etc., will be stored with the study records. These records will be made available to oversight organizations such as the US EPA, Quality Systems Office, and the IACUC. The protocol, protocol amendments, raw data, statistical analysis, tabular calculations, and graphic analysis of the data will be saved with the study records. Additionally, memoranda to the study file, study logs, signature logs, final reports, and final report amendments will be archived at USAIPH. Some ancillary records such as maintenance and calibration logs, environmental monitoring logs, animal room log books, all veterinarian staff duties logbooks, training files, etc. may be stored in the archives but not stored with the study files.

V.1.12. Study Time Frame: Estimated initiation date for the study is May 2012. Estimated completion date for the study is July 2012.

V.2. Data Analysis: For variables that are measured only at the end of the study, the dose groups will be compared using a one-factor analysis of variance (ANOVA). Organ to brain and organ to body weight ratios will be calculated and analyzed similarly to the other parameters measured at the end of the study. If the dose group effect is significant, post hoc tests will be used to compare pairs of dose groups and dose groups to the control group; a Tukey's multiple, comparison test if the variance of the

groups is similar and a Dunnett's T3 test if the variances are unequal. Variance equality will be determined by a Levene's test.

For absolute organ weights, comparison of the dose groups will be made using an analysis of covariance (ANCOVA), with body weight at the end of the study being the covariate used. Even though the dose groups will be assigned at Day 0 to keep the average weight for each dose group similar, the weights can change during the study dependent on the dose group. The ANCOVA will adjust for any differences in body weights among the dose groups at the end of the study, because heavier animals would tend to have heavier organs. If the dose group effect is significant, an appropriate post hoc test will be used to compare pairs of dose groups and dose groups to the control group.

Dose groups will also be compared with respect to absolute body weights, as well as weekly changes in body weight and net weight changes using a one-factor ANOVA. Dose groups will also be compared with respect to net food consumption for the study using a one-factor ANOVA. If the ANOVA is significant, the post hoc tests will be used to compare pairs of dose groups; a Tukey's multiple, comparison test if the variance of the groups are similar and a Dunnett's T3 test if the variances are unequal. Variance equality will be determined by a Levene's test.

Statistical analysis of the litter/pup parameters will be done by using an ANOVA test to see if the means of the four dose groups are significantly different. The data will first be checked to fit the normality and homogeneity of variance assumptions. If the ANOVA produces significant results (p-value less than or equal to .05) and the two assumptions are met, then a Tukey or Dunnett test will be used to compare the individual dose groups against each other. If the homogeneity of variance assumption is violated, a Dunnett's T3 post hoc test will be used. If the normality assumption is not met, a nonparametric Kruskal-Wallis test will be used in place of the ANOVA test.

SPSS 16.0 will be used to perform all analyses and statistical significance will be defined as $p \leq .05$ for all tests.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: The objectives of this study are to determine the initial reproductive and developmental toxicity of NTO through the use of a screening test and to confirm the effects of repeated-dose exposure to NTO using different exposure durations and dose levels than previously evaluated. The data from this study will aid in the assessment and evaluation of the toxic characteristics of the test substance. There are no appropriate animal substitutes (e.g., computer models, tissue/cell cultures) for the data that will be produced in this study. No non-animal alternative would provide the necessary toxicological information provided by this study. Therefore, it is necessary to perform this study in an animal model.

V.3.2. Animal Model and Species Justification: The test guidelines for the Organisation for Economic Co-Operation and Development (OECD) state that the rat is the preferred species (reference 6). Sprague-Dawley rats are the strain of rat that have been historically used for oral toxicity studies by USAPHC TOX and are the recommended species due to an historical and extensive database.

V.3.3. Laboratory animals

V.3.3.1. Genus and Species: *Rattus norvegicus*

V.3.3.2. Strain/Stock: Sprague-Dawley

V.3.3.3. Source vendor: Charles River Laboratories, Wilmington, MA (USDA 14-R-0144) or from animals born in-house and transferred to this protocol. If the animals born in-house are used, they will not have been previously exposed to any test material as a result of the previous protocol. A sufficient number of male and female rats will have to be available to fulfill the 100 animals required for this study, otherwise all animals will be ordered from Charles River Laboratories.

V.3.3.4. Age (at exposure): Approximately 8 weeks

V.3.3.5. Weight (at exposure): Age appropriate

V.3.3.6. Sex: Male and female (nulliparous and nonpregnant).

V.3.3.7. Special Considerations: None

V.3.4. Number of Animals Required (By Species):

Group	No. of Male Rats	No. of Female Rats
Control	10	10
31.25 mg/kg NTO	10	10
125 mg/kg NTO	10	10
500 mg/kg NTO	10	10
Weight Matching	2	2
Satellite/Recovery	20	0
Pups*	200	200
	TOTAL = 262	**TOTAL = 242**

(*) The estimation of numbers of pups was made with the assumptions that each pair would produce an average of 10 pups and the sex ratio of the offspring would be 1:1.

OECD Test Guidelines (reference 6) recommend initiating the study with 10 rats/sex/dose group with the expectation of providing at least 8 pregnant females per dose group. This is normally the minimum acceptable number of pregnant females per group to assure a meaningful evaluation of the potential of a test substance to induce

reproductive/developmental effects. "Additional" rats need to be ordered for weight matching purposes because these same guidelines require that the body weight variation for each exposure group of rats prior to exposure be within ± 20% of the mean weight of each sex.

V.3.5. Refinement, Reduction, Replacement

V.3.5.1. Refinement: Standard rat enrichment will be implemented in accordance with TOX SOP 122 (reference 12). During the co-housing period rats will be pair-housed (1 male to 1 female) for a period not to exceed 2 weeks. All animals on this study will be handled on a frequent basis and provided a form of environmental enrichment (e.g., nylabones) throughout the study period.

V.3.5.2. Reduction: This test guideline combines two individual tests (reproductive/developmental screen and repeated-dose toxicity) into one in order to reduce the number of animals needed to determine toxicity. As indicated above, an effort is made to use the fewest number of adults as possible that will produce a quantity of offspring that will enable any significant differences to be detected statistically. Tissue sharing may be allowed, however, only if doing so will not affect the validity of the study.

V.3.5.3. Replacement: No non-animal alternatives are known to exist that will provide the toxicity data required. At this time, there are no non-animal alternatives that can fully replicate the complex processes that occur within an intact mammalian organism.

V.4. Technical Methods

V.4.1. Pain/Distress Assessment:

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of Animals
NOTE: Estimates listed in Columns B-E below are modeled after a maximum number of animals including those that may be used as recovery animals.

V.4.1.1.1.1. Column B: 4 rats (2 "additional" rats of each sex ordered for weight matching purposes)

V.4.1.1.1.2. Column C: 400 pups (based on estimated average of 10 pups per parental pair and 1:1 sex ratio)

V.4.1.1.1.3. Column D: 100 rats (all adult rats on study will have cardiac blood collected while under anesthesia)

V.4.1.1.1.4. Column E: None

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Anesthesia will be administered prior to cardiac blood collection and euthanasia. Anesthesia will consist of CO_2 gas. For CO_2 anesthesia, study staff will ensure that the CO_2 tank is sufficiently full and connected to the CO_2 chamber. The rat will be placed in the CO_2 chamber, the lid put on the chamber, and the CO_2 valve turned on at a low flow (approx, ¼ turn on the tank valve). When the rat is sufficiently anesthetized (shallow breathing pattern) it will be removed from the chamber and immediately placed on a necropsy board, where prior to performing blood sampling, a proper plane of anesthesia will be ensured by the rat's lack of responsiveness to a toe-pinch.

V.4.1.2.2. Pre- and Post-procedural Provisions: A thorough physical examination of each rat will be performed by study personnel at least once per day during the pre-mating, mating, pregnancy, and lactation phases. Animals found dead will be promptly necropsied or refrigerated. Observations will be detailed and carefully recorded in the study records. Details related to observations and/or physical examination of rats and collection of rat body weights is described in Section V.1.6.

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures

V.4.1.3.1. Source(s) Searched: BRD (Biomedical Research Database), DOAC (DTIC Online Access Controlled)* Technical Reports, DOAC Research in Progress, FEDRIP, PubMed, Web of Science

V.4.1.3.2. Date of Search: 12 August 2011(updated 10 January 2012)

V.4.1.3.3. Period of Search: 1898-2011

V.4.1.3.4. Key Words of Search: (3-nitro-1,2,4-triazol-5-one or 3 nitro 1,2,4 triazol 5 one or triazole* or nitro compound*) and toxic* and (reproduc* or develop* or growth* or embryo* or fetus or fetal) and (repeat* dos* or time factor*) and (((cardiac near blood near collect*) or (heart near blood near collection*)) and (method* or technic* or techniq*)) and (alternative* or welfare or method* or model* or vitro or pain* or distress* or simulat* or video or comput* or software or cadav* or amphibian* or plastinat* or replac* or refin* or reduc*)

V.4.1.3.5. Results of Search: The literature search identified 233 references pertaining to alternatives to painful procedures, with 153 of those references pertaining to rat cardiac blood collection alone. However, no alternatives to the painful or distressful procedures (e.g., illness resulting from administration of the test substance, cardiac bleed) in this protocol or methods to relieve pain or distress without altering the outcome of the study were found. In addition, although other methods exist for blood collection from the laboratory rat, none of these alternative methods would allow the investigators to collect a large enough volume of blood to perform clinical chemistry and hematology

analysis. The use of CO_2 anesthesia is primarily based on the experience of the investigators performing the blood sampling procedure as well as the hematology and clinical chemistry analysis.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Not applicable.

V.4.2. Prolonged Restraint: N/A

V.4.3. Surgery: None

V.4.3.1. Pre-Surgical Provisions: N/A

V.4.3.2. Procedure: N/A

V.4.3.3. Post-Surgical Provisions: N/A

V.4.3.4. Location: N/A

V.4.3.5. Surgeon: N/A

V.4.3.6. Multiple Major Survival Operative Procedures: None

V.4.3.6.1. Procedures: N/A

V.4.3.6.2. Scientific Justification: N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections: None

V.4.4.2. Biosamples: All animals placed on study will undergo blood collection (approximately 3-6 ml of blood) just prior to euthanasia. All blood sampling will occur under CO_2 gas anesthesia via cardiac puncture. An appropriate size needle (18-21 gauge, 1-1.5 inch needle, depending on the size of the rat) will be fitted onto a 3-10 ml syringe and inserted anteriorly under the xiphoid region of the rat at an approximate 45° angle and advanced firmly through the diaphragm and into the heart. Slight negative pressure should be placed on the syringe plunger and the required amount of blood withdrawn from the rat. Following collection of the blood sample, the needle should be slowly withdrawn from the rat. To minimize blood hemolysis, the needle should be removed from the syringe, the microtube top removed, and the blood sample discharged directly into the microtube tube directly from the syringe. The microtube will then be recapped. EDTA tubes need to be inverted gently several times immediately after introducing blood into the tube. Blood collection will be promptly followed by euthanasia as described in Section V.4.6.

V.4.4.3. Adjuvants: N/A

V.4.4.4. Monoclonal Antibody (MAbs) Production: N/A

V.4.4.5. Animal Identification: Animals will be identified by cage cards according to TOX SOP 003 (reference 13). An identification number (e.g., the last 3 digits of the animal number) will also be marked on the tail of each rat with a water-insoluble marker in order to ensure proper identification of rats when removed from their cages or pair-housed during mating.

V.4.4.6. Behavioral Studies: N/A

V.4.4.7. Other Procedures: N/A

V.4.4.8. Tissue Sharing: Tissue sharing may be allowed upon request provided there is no effect on the validity of the study or change any euthanasia methods as stated in the protocol.

V.4.5. Study Endpoint: The study endpoint is euthanasia at scheduled time periods. The scheduled euthanasia for male rats will follow blood collection at the conclusion of the 28-day (prior to and during co-housing) dosing period and for female rats following blood sampling on postnatal day 4 or 5. The scheduled euthanasia for all satellite/recovery animals (if used) will be following blood collection at the conclusion of the recovery period (14 days to one month). Though no illness or distress is expected from the administration of NTO nor from any of the procedures, any animal considered moribund will be humanely euthanized as described in Section V.4.6. One or more of the clinical signs will be considered to be indicative of a moribund animal: impaired ambulation (e.g., impairment prevents animals from reaching food/water for greater than 18 hours); excessive weight loss or emaciation (e.g., ≥ 20% body weight loss as compared to the start of the study); prolonged labored breathing (e.g., lasting longer than 8 hours and accompanied by extreme lethargy); continuous seizure activity (e.g., lasting longer than 1 hour); inability to urinate or defecate (e.g., inability lasts greater than 24 hours); decreased body temperature (e.g., body temperature remains below 98°F for more than 2 hours); prolonged inability to move into the upright position (e.g., inability to upright lasts for more than 2 hours).

V.4.6. Euthanasia: Adult rats: after blood has been collected, the rats will be returned to the CO2 chamber and gas will continue for a few minutes beyond the time the rats appear dead (no respirations or movements for greater than 2 minutes, eyes fixed, and mucus membranes and skin are cyanotic). Death of all adult rats will be ensured with a thoracotomy as part of the necropsy procedure.

Pups will be placed in the CO2 chamber allowing adequate floor space for each pup. The gas will be turned on at a slow flow until the pups appear anesthetized (no movements except breathing) then the CO2 may be administered at a faster flow rate until the pups appear dead (no respirations or movements for greater than 2 minutes). Decapitation with sharp scissors will be used to ensure that all pups are dead. Study staff will euthanize the animals.

V.5. Veterinary Care

V.5.1. Husbandry Considerations: The animals will be housed in plastic, solid-bottom shoebox cages (size appropriate to the body weight of the rat) and given water and certified rodent feed *ad libitum* during the study (with exception of overnight fasting prior to necropsy). During the 2-week co-housing period, rats will be pair-housed (1 male to 1 female) in shoebox cages with an elevated wire rack (no bedding) which will allow investigators to check for the presence of a sperm plug in the bottom of the cage. Rats will be single-housed for the study period, including acclimation, except during the 2-week co-housing period. Animal rooms will be maintained according to the conditions specified in TOX SOP 004 (reference 14). All rats will undergo a 5-day acclimatization period. Body weight and observation data may also be collected for rats by study personnel during the acclimation period in an attempt to more accurately monitor the health status of the rats in preparation for their use on study. However, animals will not be weighed or handled by study personnel within the first 24 hours after their arrival to the facility.

V.5.1.1. Study Room: Studies will be conducted at the USAIPH Toxicology Portfolio animal facility, Bldg E-2100 or Bldg E-2101, study room as assigned. The animal facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

V.5.1.2. Special Husbandry Provisions: General husbandry procedures performed by the animal care staff (e.g. cage changes) will need to be performed following morning dosing/observations during the co-housing, pregnancy, and lactation phases. Additional nesting enrichment may be provided to pregnant females at the discretion of the Attending Veterinarian.

V.5.1.3. Exceptions: Not Applicable.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: All animals will be observed at least once daily by assigned Veterinary Medicine personnel for husbandry conditions, humane care, and general health. If dead animals are found they will immediately be necropsied or refrigerated.

V.5.2.2. Emergency Veterinary Medical Care: Veterinary care is available 24 hours a day, 7 days a week In the case of an emergency health problem, if the PI or co-PI is unavailable or if the investigator staff and veterinary staff cannot reach consensus on treatment, the veterinarian has the authority to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary. If the veterinarian orders treatment or euthanasia after phone consultation and the PI does not concur, a veterinarian will conduct a physical examination. To facilitate communication, the vet med staff will maintain an emergency contact roster in the vet tech office. In an emergency, the veterinary staff will phone the

numbers (office, home, and mobile) listed for the PI and co-PI. If the PI or co-PI cannot be reached by phone within 15 minutes, then they are considered unavailable.

V.5.3. Environmental Enrichment

V.5.3.1 Enrichment Strategy: All enrichment will be provided in accordance with TOX SOP 122 (reference 12). Animals will be handled on a frequent basis and provided a form of environmental enrichment (e.g., nylabones) throughout the study. Additional nesting enrichment may be provided to pregnant females at the discretion of the Attending Veterinarian.

V.5.3.2. Enrichment Restriction: All rats will need to be singly housed except during the 2-week co-housing period. Cylindrical retreats will not be placed in the cages during the co-housing, pregnancy, and lactation phases.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Staff Member	Procedure	Training	Experience	Qualifications
Lee Crouse	Oral gavage, observations, handling, blood collection, CO_2 euthanasia, anesthesia, necropsy	Humane Care & Use of Lab Animals (May 2000); Rodent Handling Techniques, WRAIR (includes oral gavage in rats; Nov 1996); Rat handling, gavage, injections, blood collection (July 2007); Rat cardiac bleeding under isoflurane (Dec 2008, May 2009); necropsy (Oct/Dec 2007)	16+ Yrs Animal Research	M.S., Environmental Science
Emily Lent	Oral gavage, observations, handling, blood collection, CO_2 euthanasia, necropsy	Rat handling, gavage, injections, blood collection (July 2007); Rat bleeding techniques & tissue collection (Apr 2008); necropsy (Jul/Oct 2007, Apr 2008); Rat oral gavage (March 2008); Oral gavage in rats (May 2009)	11+ Yrs Animal Research	M.S., Wildlife Biology; Ph.D., Natural Resources and Environmental Studies
Mike Quinn	Oral gavage, observations, handling, blood collection, CO_2 euthanasia, necropsy	Necropsy (May 2005, Oct 2007, Dec 2009); Oral gavage (March 2008); Rodent Handling Workshop (June 2005)	13+ Yrs Animal Research	Ph.D., Animal Science
Mark Way	Observations, handling, CO_2 euthanasia, Necropsy	Rodent & Small Animal Handling workshops (2003, 2007); necropsy (May 2007)	17+ Yrs Animal Research	B.S., Biology; LAT
Art	Observations,	Inhalation testing	30+ Yrs	B.S., Biology;

O'Neill	handling, CO_2 euthanasia, necropsy	experience (memo from DuPont dated Oct 2008); necropsy (Dec 2007)	Animal Research	LATG
Terry Hanna	Observations, handling, CO_2 euthanasia, necropsy,	Rodent Handling & Techniques (1992); Rodent & Small Animal Handling Workshop (2004, 2005, 2006); Rat handling and gavage (2007), rat euthanasia via CO_2 with thoracotomy (3/2009); rat isoflurane anesthesia, cardiac blood draw, & CO_2 euthanasia (2009); necropsy (2009, 2010); Functional observation battery (FOB) training (5/2007, 8/2008, 1/2009); Acoustic Startle Response (handheld clicker & startle chamber operations) (1/2009)	15+ Yrs Animal Research	ALAT
Will McCain	Observations, handling, necropsy	Animal Care & Use Training (Mar 1995); Humane Care & Use of Lab Animals (May 2000); necropsy (Dec 2007, Feb/Dec 2008, Feb 2009)	30+ Yrs Animal Research	Ph.D., Toxicology
Alicia Shiflett	Observations, handling, necropsy	Rodent handling & techniques training; observations, handling/restraint, weighing, basic bleeding (Nov 2008); rat CO_2 euthanasia with thoracotomy (Mar 2009); rat necropsy & tissue collection (Mar 2008, Jan 2010)	2+ Yrs Animal Research	Associates Degree, Histology/Science

VII. BIOHAZARD/SAFETY:

In accordance with PHC Reg. 385-1, CHPPM Reg. 385-5, and TOX SOP 083, standard laboratory protection (e.g., safety glasses, gloves or eye protection, lab coat) shall be used when handling the neat test substance. The test substance shall be stored in a sealed container at room temperature when not in use. The test substance will be handled in a laboratory fume hood when necessary. Although the precise toxicity of the test substance may not be known, information regarding its chemical family will be provided so that a reasonable assessment of its safety can be made (references 15, 16, and 17). While in animal rooms or while handling animals in a laboratory, personnel will wear appropriate PPE (e.g., lab coat, gloves, face mask).

VIII. ENCLOSURES:

A. References

IX. ASSURANCES:

IX.1. As the Study Director/ Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

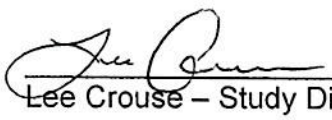
D. Biohazard/Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/ observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical, and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL and WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.



Lee Crouse – Study Director (PI)

20120329
Date (YYYYMMDD)

IX.2. As the Primary Co-Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as the Primary Co-Investigator, I am authorized and responsible for performing all procedures and manipulations as assigned to the SD/PI in the SD/PI's absence. This includes euthanasia of distressed animals.

C. Training: I verify that I am technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

D. Responsibility: I acknowledge the inherent moral, ethical, and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

E. Painful Procedures: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL and WILL NOT be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

Emily May Lent — Primary Co-Investigator

20120328
Date (YYYYMMDD)

IX.3 ASSURANCES: As a Co-Investigator on this protocol, I provide the following assurances:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Authority: I understand that, as a Co-Investigator, I am authorized, responsible for, and willing to perform all procedures and manipulations as assigned to me by the SD/PI.

C. Training: I verify that I am technically competent and have been or will be properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the assigned procedures/manipulations performed by me.

D. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that I will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to participate in this study in the spirit of the fourth "R", namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible, and conducting humane and lawful research.

E. Painful Procedures: I am participating in biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. I will follow the direction of the SD/PI relative to potential pain and/or distress and relief by the use of anesthetics, analgesics and/or tranquilizers.

Michael J. Quinn Michael J. Quinn 3/29/12

(PRINT) (Signature) (Date)
First name, MI, Last name of Co-Investigator

(PRINT) (Signature) (Date)
First name, MI, Last name of Co-Investigator

(PRINT) (Signature) (Date)
First name, MI, Last name of Co-Investigator

(PRINT) (Signature) (Date)
First name, MI, Last name of Co-Investigator

APPENDIX A

REFERENCES

1. Spear, R.J., Louey, C.N., and Wolfson, M.G. A Preliminary Assessment of NTO as an Insensitive High Explosive. DSTO Materials Research Laboratory, Maribyrnong, Victoria 3032, Australia, MRL-TR-89-18, 1989.

2. Smith, Matthew W. and Cliff, Matthew D. NTO-Based Explosive Formulations: A Technology Review. Weapons Systems Division, DSTO Aeronautical and Maritime Research Laboratory, Salisbury South Australia 5108 Australia, DSTO-TR-0796, 1999.

3. Technical Report, U.S. Army Public Health Command (Provisional) [USAPHC (Prov)] [formerly U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM)], L.C.B. Crouse, et al., 2009, Subchronic Oral Toxicity of NTO in Rats.

4. Duncan, Kendal. 2002. Insensitive Munitions and the Army: Improving Safety and Survivability. Army Logistician PB700-02-1, Volume 34, Issue 1: 16-17.

5. Davies, P.J. and Provatas, A. Characterisation of 2,4-Dinitroanisole: An Ingredient for Use in Low Sensitivity Melt Cast Formulations. Defence Science and Technology Organisation, PO Box 1500, Edinburgh, South Australia 5111 Australia, DSTO-TR-1904, 2006.

6. Organisation for Economic Co-Operation and Development (OECD) Section 4 (Part 422): Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test. Guideline for the Testing of Chemicals (22 March 1996).

7. USAPHC AIPH SOP No. DLS 801, Chromatographic\Spectrophotometric Analysis of Toxicology Directorate Samples, 2012.

8. USAPHC, AIPH, Toxicology Portfolio SOP No. CP034-003, Clinical Chemistry Analysis of Blood Specimens, 2011.

9. USAPHC, AIPH, Toxicology Portfolio SOP No. CP001-002, Cell-Dyn 3700 Hematology Analyzer, 2011.

10. Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards.

11. Guide for the Care and Use of Laboratory Animals, National Academies Press, National Research Council, 2011.

12. USAPHC, AIPH, Toxicology Portfolio SOP No. AP122.003, Animal Environmental Enrichment, 2012.

13. USAPHC, AIPH, Toxicology Portfolio SOP No. AP003.002, Individual Animal Identification, 2011.

14. USAPHC, AIPH, Toxicology Portfolio SOP No. AP004-P-001, Animal Health Technician Duties within the Animal Facility, 2009.

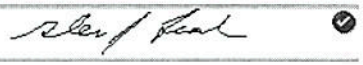
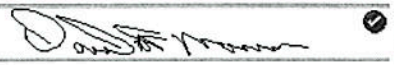
15. USAPHC Regulation 385-1, Safety and Occupational Health Program, 15 January 2010.

16. USACHPPM Regulation 385-5, Occupational Health and Safety of Animal Users, 1 June 2007.

17. USAPHC, AIPH, Toxicology Portfolio SOP No. GL083-P-002, Health and Safety of Laboratory Personnel, 2010.

PROTOCOL REVIEW, SUPPORT, APPROVAL SHEET

PROTOCOL NUMBER:	TITLE: Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat
0FP4 - 93 - 12-03-03 SUB-JONO TEST TYPE IACUC NUMBER	

1. SCIENTIFIC MERIT (PEER REVIEW)			
1a. Printed Name (First, MI, Last) Dr. Desmond I. Bannon	1b. Title Toxicologist	1c. Signature BANNON.DESMOND.I.1255711420	1d. Date (yyyy/mm/dd) 20111230
2. DIRECTOR			
2a. Printed Name (First, MI, Last) COL Chris E. Hanson	2b. Title Director, Toxicology Portfolio	2c. Signature HANSON.CHRIS.E.1149169065	2d. Date (yyyy/mm/dd) 20111220
3. PROGRAM MANAGER			
3a. Printed Name (First, MI, Last) Dr. Glenn J. Leach	3b. Title Toxicology Portfolio Manager	3c. Signature 	3d. Date (yyyy/mm/dd) 20111221
4. ATTENDING VETERINARIAN			
4a. Printed Name (First, MI, Last) MAJ Dawn C. Fitzhugh	4b. Title Attending Veterinarian, USAPHC	4c. Signature FITZHUGH.DAWN.CATHERINE.1036926129	4d. Date (yyyy/mm/dd) 20120103
5. ANALYTICAL CHEMISTRY (If Applicable)			
5a. Printed Name (First, MI, Last) David F. Morrow	5b. Title Laboratory Services Consultant	5c. Signature 	5d. Date (yyyy/mm/dd) 20120103
6. SAFETY MANAGER			
6a. Printed Name (First, MI, Last) Roy A. Valiant	6b. Title Safety Manager, USAPHC	6c. Signature VALIANT.ROY.A.1081780591	6d. Date (yyyy/mm/dd) 20120118
7. STATISTICIAN (If Applicable)			
7a. Printed Name (First, MI, Last) Karen D. Deaver	7b. Title Statistician	7c. Signature DEAVER.KAREN.DEVILBISS.1400519672	7d. Date (yyyy/mm/dd) 20111229

PROTOCOL NUMBER: 0FP4 - 93 - 12-03-03 (SUB-JONO / TEST TYPE / IACUC NUMBER)	TITLE: Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat		
8. SIO-QAT (GLP COMPLIANCE AND QA SUPPORT)			
8a. Printed Name (First, MI, Last) Michael P. Kefauver	8b. Title Quality Systems Office	8c. Signature KEFAUVER.MICHAEL.P.1229209678	8d. Date (yyyy/mm/dd) 20120124
9. CHAIRMAN, IACUC			
9a. Printed Name (First, MI, Last) Kristin T. Newkirk	9b. Title Animal Care & Use Specialist, IACUC Chairperson	9c. Signature NEWKIRK.KRISTIN.TORELL.1014786895	9d. Date (yyyy/mm/dd) 20120330
10. INSTITUTIONAL OFFICIAL			
10a. Printed Name (First, MI, Last) John J. Resta	10b. Title Director USAIPH	10c. Signature RESTA.JOHN.J.1229129305	10d. Date (yyyy/mm/dd) 20120330
11. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR			
11a. Printed Name (First, MI, Last) Lee C. Crouse	11b. Title Study Director	11c. Signature CROUSE.LEE.1239523263	11d. Date (yyyy/mm/dd) 20120402
12. OTHER ORGANIZATION(S) PROVIDING SUPPORT (AS NEEDED):			
12a. Printed Name (First, MI, Last)	12b. Title	12c. Signature	12d. Date (yyyy/mm/dd)
13. STUDY SPONSOR:			
13a. Printed Name (First, MI, Last)	13b. Title	13c. Signature	13d. Date (yyyy/mm/dd)

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 20120402	2. PROTOCOL NUMBER: 0FP4-93-12-03-03	3. MODIFICATION#: 1

4. PROTOCOL TITLE: Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Lee Crouse	410-436-5088	MCHB-IP-TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 14 | 1b. N/A ☐

2. ORIGINAL PROTOCOL TOTAL: 504 (including estimated pups)				3. PROTOCOL TOTAL AFTER MODIFICATION: 518 (including estimated pups)				
2a. USDA pain cat:	B: 4	C: 400	D: 100	E.	3a. USDA pain cat:	B: 18	C: 400	D: 100	E:

4. Yes	No	
☐	☒	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☐	☒	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

SECTION III. MODIFICATION/JUSTIFICATION

Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals

PROTOCOL Page, paragraph, section: Page 11, V.3.3.3, V.3.4.

1. MODIFICATION:

As stated in this protocol, attempts are being made to use animals born in-house for this study. A total count of all pups that can be transferred from protocol number 0FP3-95-12-02-01 (SD Emily Lent) to this protocol is now available. This protocol requires the use of 62 adult male and 42 adult female Sprague-Dawley rats; however 66 male pups and 52 female pups are available for transfer from protocol number 0FP3-95-12-02-01 when they reach postnatal day 21 (approximately 3 April). Taking all the available extra pups will initially result in an increase of 14 animals for this protocol while they are being raised to an age that they can be utilized. Sixty-two male and 42 female rats will be selected for use when the rats reach approximately 8 weeks of age. All remaining animals will then be transferred to the training protocol or humanely euthanized at the discretion of the Attending Veterinarian.

1a. JUSTIFICATION/REASON:

The pups transferred from protocol number 0FP3-95-12-02-01 will be held for approximately the next 5 weeks until they reach an age that they can be utilized for this study. Breeding for this reproductive/developmental screening test requires pairing unrelated animals (outbreeding) and keeping all of the animals available for transfer to this protocol maximizes the ability to ensure outbreeding. In addition, if 62 adult male and 42 adult female rats of similar weight range are not available when the pups reach 8 weeks of age, the entire group of transferred animals would need to be humanely euthanized and a separate order placed with Charles River Laboratories. Keeping all of the pups at this time increases the chance for success with this reduction effort and helps to ensure that the study is initiated on time.

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
Page 12, V.4.1.1.1.1.	2. MODIFICATION: Column B animals increases from 4 rats (2 male, 2 female) to 18 rats (6 male, 12 female).
	2a. JUSTIFICATION/REASON: The animals being used for this study are extra pups that were born to timed-pregnant females for another study and were not needed. There were enough extra pups born to supply all the animals needed for this study at one time, plus there are 14 extra. We would like to hold these extra 14 rats as category B animals to ensure there will be sufficient animals of similar weight range to run the study.
Page 15, V.5.1.	3. MODIFICATION: All pups transferred to this protocol will be group-housed (same sex) in appropriately-sized shoebox cages for the 5-week period that they are held prior to study initiation. They will be separated and single-housed just prior to study initiation when they reach approximately 8 weeks of age.
	3a. JUSTIFICATION/REASON: The pups will only be 3 weeks of age when transferred to the protocol and group-housing will allow the pups to acclimate to solid food and the automatic watering system more efficiently.
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Lee Crouse	Signature	DATE: (yyyy/mm/dd) 20120409
2. PROGRAM MANAGER:: (Printed Name) Shannon Wallace	Signature	DATE: (yyyy/mm/dd) 2012 0409
3. ATTENDING VETERINARIAN: (Printed Name) Dawn Fitzhugh	Signature	DATE: (yyyy/mm/dd) 2012 0409
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE)	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) KRISTIN T. NEWKIRK	APPROVED REVIEWED YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 2012/04/09

USACHPPM PROTOCOL MODIFICATION

For use of this form, see DTOX SOP 085

1. DATE: (YYYY/MM/DD) 2012/05/09	2. PROTOCOL NUMBER: 0FP4-93-12-03-03	3. MODIFICATION#: ~~2~~ GLP-1 MAK 05/16/12 (1)

4. PROTOCOL TITLE: Repeated-Dose and Reproductive/Developmental Toxicity of NTO in the Rat

5. STUDY DIRECTOR/PRINCIPAL INVESTIGATOR:	6. WORK PHONE:	7. OFFICE SYMBOL:
Lee Crouse	410-436-5088	MCHB-IP-TEP

SECTION I. PREVIOUSLY APPROVED AND CURRENTLY IN USE PROTOCOL MODIFICATIONS:

1. MODIFICATION NUMBER	2. SHORT DESCRIPTION OF PRIOR APPROVED MODIFICATION(S)	3. NO. & SPECIES OF ANIMAL REQUESTED	4. APPROVED DATE (XX XXX XXXX
1	Retention of 14 additional animals transferred from protocol 0FP3-95-12-02-01 until study initiation.	14 additional Sprague-Dawley Rats	9 Apr 2012

SECTION II. CHANGE IN TOTAL # OF ANIMALS USED AND/OR CHANGE IN USDA PAIN CATEGORY

1a. CHANGE: INCREASE TOTAL APPROVED ANIMALS BY: 1b. N/A ☒

2. ORIGINAL PROTOCOL TOTAL: 518 (including estimated pups) 3. PROTOCOL TOTAL AFTER MODIFICATION: 518 (including estimated pups)

2a. USDA pain cat:	B: 18	C: 400	D: 100	E.	3a. USDA pain cat:	B: 18	C: 400	D: 100	E:

4. Yes	No	
☐	☒	Modification requires specific changes or additions to the experimental design of the protocol. (Section V.I. of the template.)
☒	☐	Modification requires changes to the technical methods, i.e., procedures, routes of administration, biosample collection, etc. (Section V.4. of the protocol template.) Indicate training of personnel for new methods, procedures being used.
☐	☒	Modification requires additions or changes in personnel performing procedures. (Section VI of the protocol template.) Include training and qualification information and tasks that each individual will be performing. If changing the Study Director/PI, a signed Assurance Statement needs to be submitted with the modifications.

PROTOCOL Page, paragraph, section	SECTION III. MODIFICATION/JUSTIFICATION *Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals*
Page 14, V.4.4.2	1. MODIFICATION: Sperm analysis will be performed on male animals from all dose groups, including the satellite recovery group. One of the epididymides taken from each of the male rats will be transferred to a member of the study staff immediately after obtaining the weight and used to perform sperm analysis.
	1a. JUSTIFICATION/REASON: Based on the history of NTO to induce reproductive effects in male rats at moderate doses, sperm analysis will add to a meaningful interpretation of the data obtained from this reproductive/developmental screening test.

(1) GLP only modifications need to be labeled GLP-1, GLP-2, etc. to differentiate from IACUC Modification

PROTOCOL Page, paragraph, section	*Explain the modification indicated above in the area below. Indicate any changes to the 3R's (Refinement, Reduction, Replacement) resulting from changes in number of animals used.*
Page 8, V.1.9	2. MODIFICATION: In addition to the observations and tissues listed in the gross necropsy section of the approved protocol, the prosector will also be counting the number of implantation sites and corpora lutea.
	2a. JUSTIFICATION/REASON: OECD Guideline 422 recommends counting the number of implantation sites and corpora lutea but only states the recommendation in the reporting results section. Although this does not involve any additional tissue removal or weighing, it should be added to the gross necropsy section to account for the additional data that will be added to the gross necropsy sheet.
	3. MODIFICATION:
	3a. JUSTIFICATION/REASON:
	4. MODIFICATION:
	4a. JUSTIFICATION/REASON:

Continued on next page YES ☐ NO ☒

SECTION IV. SIGNATURES AND DATES

1. STUDY DIRECTOR: (Printed Name) Lee Crouse	Signature	DATE: (yyyy/mm/dd) 20120514
2. PROGRAM MANAGER:: (Printed Name) LTC Shannon Wallace, DVM, DACVP	Signature	DATE: (yyyy/mm/dd) 2012 0515
3. ATTENDING VETERINARIAN: (Printed Name) N/A	Signature	DATE: (yyyy/mm/dd)
4. CHPPM SAFETY OFFICER/OCC HEALTH REP: (IF APPLICABLE) N/A	Signature	DATE: (yyyy/mm/dd)
5. CHAIR, IACUC OR QA (If no animal related changes): (Printed Name) QA, Michael P. Kefauver	APPROVED (REVIEWED) YES ☒ NO ☐ Signature	DATE: (yyyy/mm/dd) 20120516